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Effect of pH, Fat Level, and Various Browning Agents on Composition, Color, Texture, and Sensory Characteristics of Dark-Cutting Beef Patties

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EFFECT OF PH, FAT LEVEL, AND VARIOUS BROWNING AGENTS ON COMPOSITION, COLOR, TEXTURE, AND SENSORY CHARACTERISTICS OF DARK-CUTTING BEEF PATTIES

by

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ABSTRACT

Effect of pH, Fat Level, and Various Browning Agents on Composition, Color, Texture, and Sensory Characteristics of Dark-Cutting Beef Patties

by

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Extra lean (3.3% fat) and lean (20.0% fat) hamburgers in three pH groups (≤ 6.0; 6.01-6.49; 6.50-6.92) were evaluated for cooking-temperature profile, total process lethality, and physical properties after cooking to 71°C by double-side frying on an electric grill. Neither cooking-temperature profile nor cooking time was affected by hamburger fat content or pH. Double-side frying to 71.1°C internal temperature was adequate for more than 6-log destruction of viable *E. coli* O157:H7 and *Salmonella* at the geometrical center of extra lean and lean hamburgers. The coldest spot was on the circumferential surface, as indicated by the presence of a red ring of undenatured myoglobin, and confirmed by the finite-element temperature distribution model.

The effect of pH (5.80, 6.29, 6.73) on myoglobin denaturation in extra lean (3.3% fat) and lean (20.0% fat) hamburgers was studied. Compared to normal meat (pH = 5.8),

raw extra lean ground beef of pH = 6.73 had significantly lower oxidation-reduction potential (ORP) value, lower concentration of metmyoglobin after 48 hr of refrigerated storage, and more distinct cherry-red color. Percent of myoglobin denaturation during cooking was affected mainly by pH and was not affected by total pigment or fat content of hamburgers. A pH $\geq$ 6.5 and ORP $\leq$ -200 mV were characteristic of dark-cutting beef.

In a third experiment, extra lean (3.5%) and lean (20.0%) beef patties were made from normal beef (pH = 5.70) and dark-cutting beef (pH = 6.60). Controls were made with no additives or with 1% salt and 10% added water. Various browning agents (1% glucose, 0.2% caramel colorant, 0.3% calcium peroxide, or 2.5% encapsulated lactic acid) were added with 10% water and 1% salt. Salt had a pronounced prooxidant effect on myoglobin. Distinctive absorption peaks at 541-548 nm and 577-582 nm indicated that the undenatured pigment in cooked patties was oxymyoglobin. Dark-cutting patties had more rubbery texture and slightly perceptible off-flavor. Patties with lactic acid were less juicy and had lower intensity of beef flavor than other patties, and moderate intensity of sour off-flavor. Addition of salt and encapsulated lactic acid to beef patty formulation could solve the problem of hard-to-cook patties.

(249 pages)

DEDICATION

This work is dedicated to the American people, who like to eat hamburgers.

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Igor V. Moiseev

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CHAPTER I

INTRODUCTION AND OBJECTIVES

INTRODUCTION

In the last decade, hundreds of cases of foodborne illnesses from uncooked beef hamburgers were reported. *Escherichia coli* O157:H7 was responsible for most of the outbreaks. The quality of a thermal process is determined by the temperature and duration of cooking. The characteristics of a suitable thermal process may be estimated based on the heat resistance of the target microorganism and knowledge of the temperature history during processing. Thermal resistance of *E. coli* O157:H7 can be described by the D-value and z-value, which depend on product composition (Line et al., 1991). The finite-element method (FEM) has been successfully used to model heat transfer in meat products (Puri and Anantheswaran, 1993). For both consumers and food service, cooking guidelines suggest that beef hamburgers be cooked to 71.1°C or until no pink color remains in the center, and juices are clear (USDA, 1993). However, undenatured myoglobin and oxymyoglobin may be present in sufficient concentration to cause red color in beef cooked to 71°C, if meat pH is greater than 6.0 (Trout, 1989).

Hard-to-cook hamburgers from bull meat are characterized by persistent internal red color after cooking and are associated with high pH raw meat (Mendenhall, 1989). Dark-cutting beef does not bloom and has ultimate pH above 6.0 (Tarrant, 1987). The incidence of dark-cutting carcasses with high pH is estimated at 3.2-5.2% in Ireland

the USA (Munns and Burrell, 1966). This condition is caused by an absence of glycogen in the muscles at death (Apple et al., 1995). Trout (1989) confirmed that the pH effect was the greatest at lower temperatures (55 and 62°C) where the percent denatured myoglobin was 3 to 14 times greater at pH 5.5 than at pH of 7.0. It is apparent that under identical cooking conditions, considerable variation in color may occur.

Oxidation status of myoglobin and choice of commercial beef patty formulation appears to have an effect on the amount of myoglobin denatured by 71, 81, or 87°C internal cooking temperature (Van Laack et al., 1996). Increasing the sodium chloride concentrations up to 3.0% increased the rate of metmyoglobin formation in raw ground beef (Trout, 1990). In addition to salt, various other additives might increase browning of cooked patties, including sugars for Maillard browning, caramel colorant, lactic acid, or calcium peroxide. The products of the Maillard reaction were effective inhibitors of lipid oxidation in ground pork patties (Bedinghaus and Ockerman, 1995). Denaturation of meat pigments could be increased by lowering the pH of meat products (Janky and Froning, 1973). By addition of encapsulated lactic acid to dark-cutting beef, normal pH could be achieved. Calcium peroxide has been used in dough conditioning formulations for many years (Tieckelmann and Steele, 1991). About 0.25-0.5% of CaO_2 is used in flour formulations. Beef roasts from dark-cutting meat were softer, more tender, and juicier than from normal beef (Hawrysh et al., 1985). The high pH confers a greater water-holding capacity that significantly increases cooked yield.

OBJECTIVES OF THIS STUDY

The first objective of this work was to determine physical parameters, effect of fat content, and meat pH on thermal process microbial lethality of hamburgers cooked to 71°C internal temperature by double-side frying on an electric grill.

The second objective of this study was to determine effect of total meat pigment, oxidation-reduction potential, and total reducing ability of raw ground beef on percent myoglobin denaturation by frying of 3% and 20% fat hamburgers of pH 5.6-6.9 to 71°C internal temperature.

The third objective of this study was to determine if beef patty formulations with salt, glucose, caramel colorant, calcium peroxide, or encapsulated lactic acid can reduce pink discoloration in dark-cutting beef patties cooked to 71°C.

The forth objective was to evaluate sensory and physicochemical characteristics of dark-cutting patties and normal pH beef patties with browning agents.

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CHAPTER II

LITERATURE REVIEW

DARK-CUTTING BEEF AND RELATED DARK COLOR PROBLEMS

It has been estimated that dark-cutting meat cost the beef industry approximately $132.5 million in 1991, or approximately $5 for every steer and heifer slaughtered (Smith et al., 1992; Grandin, 1992; Nunes, 1992). Consumer demands for lean meat and rising production have stimulated interest in the production of beef from bulls, though they are more likely to produce dark-cutting beef than steers. The dark-cutting or "DFD" term is derived from the dark, firm, dry appearance of meat with a high ultimate pH. This condition is caused by an absence of glycogen in the muscles at death. Muscle glycogen breakdown results from strenuous physical exercise, and also, in many species, from trauma and, possibly, psychological stress. Beef is dark-cutting when the final pH value of the meat is above 6.0 (Tarrant, 1987). In general, the major muscles of the loin and round are the most severely affected and have the highest pH of 13 major muscles (Tarrant and Sherington, 1980). The muscular activity is one of the main causes of the high ultimate pH in hindquarter muscles of dark-cutters. At pH of 5.5, normal meat contains about 1% of lactic acid, which depresses mitochondrial metabolism, causing meat to "bloom." The main pigment of meat, myoglobin, after exposure to oxygen transforms to oxymyoglobin, which gives the normal bright red color to meat. In the case of dark-cutting beef, mitochondria in myofibrils are still

active at high pH and consume surface oxygen, keeping myoglobin deoxygenated, and preventing development of normal red color (Cornforth and Egbert, 1985). Dark-cutting beef will bloom, when chilled in air or oxygen to 3°C, but will stay dark red at room temperature (Egbert and Cornforth, 1986). If mitochondrial respiration is inhibited, myoglobin of dark-cutting beef muscle is oxygenated, and meat will turn red. Similarly treated pre-rigor beef muscle remained dark (Cornforth and Egbert, 1985). Pre-rigor beef muscles have higher mitochondrial activity than dark-cutting muscles, and remain dark at relatively lower temperatures or higher oxygen concentration. The dark-cutting condition worsens progressively in meat of pH value above 6.0. The surface of such meat is usually sticky, and this stickiness and dark color are generally considered undesirable characteristics. Good color in meat, while it may not affect its nutritive value, is generally recognized and demanded by the consumer. The consumer associates dark-colored beef with old animals or spoilage (Lynch et al., 1986). For this reason retailers cannot sell fresh dark-cutting beef directly to customers. Shelf-life of fresh dark-cutting beef is shorter than normal meat. In high pH dark-cutting beef, lactic aid is reduced in concentration and glucose is absent, and consequently putrefactive bacterial growth is facilitated (Newton and Gill, 1981). In the absence of glucose, meat proteins are attacked without delay and spoilage odors can be detected when bacterial densities are still low. The elevated pH is not the main factor inducing spoilage (Newton and Gill, 1981). Because of these abnormal spoilage characteristics, dark-cutting beef cannot be successfully vacuum packaged and should instead be

preserved by freezing (Tarrant, 1987). Products made from DFD beef could retain the putrid odors of the raw meat. Addition of glucose to DFD meat and vacuum packaging under CO_2 can delay microbial spoilage (Gill and Penney, 1986; Newton and Gill, 1981).

Utilization of dark-cutting beef is a problem to meat producers and processors. The incidence of dark-cutting carcasses with high pH is estimated at 3.2-5.2 % in Ireland (Tarrant and Sherington, 1980), 8% in Canada, and 0.33% and 4.7% by several investigators in the USA (Munns and Burrell, 1966). Unusually high incidence (up to 20%) of DFD beef can occur in the fall season (Munns and Burrell, 1966). An average between 40 and 60% incidence of dark-cutting beef is found as a result of pre-slaughter treatment according to the requirements of Bulgarian State Standard No 837/85 of calves in Black-White, Bulgarian Brown, Simmental, and Hereford breeds (Alexandrova et al., 1995). Franc at el. (1988) indicated that dark-cutting beef is primary caused by agonistic, physically exhausting activities such as mounting, and further modified by stress-inducing social interactions before slaughtering. Incidence of dark-cutting depends on the quality of the animal. Munns and Burrell (1966) found on average 12% dark-cutting in commercial steers versus 3.5% in choice steers, which have higher tissue glycogen levels. Dark-cutting phenomena can be simulated by adrenaline treatment of animals; however, the pH pattern in beef carcasses suggests a different physiological mechanism of ultimate pH development (Tarrant and Sherington, 1980). Some beef producers try to measure pH in the carcasses 2 days after slaughter to identify dark-cutting beef before vacuum packaging, especially bull

beef (Tarrant, 1987). Most of the meat-packing plants do not control pH in beef carcasses. As a result of this, retailers and meat processors from time to time receive vacuum packaged dark-cutting beef. The surface of such meat is usually sticky, and this stickiness and dark color are generally considered undesirable characteristics in red meat. Consumers, used to buying bright red meat, associate this color with good quality (Smith, 1981). Meat color is one of the most important factors for consumers (Kropf, 1980). This may be because consumers are suspicious of any muscle color abnormalities (Romans and Ziegler, 1977). Dark-cutting beef is normally ground and mixed with normal beef for production of hamburgers. Sometimes, hamburgers from DFD meat stay red or pink inside, even if required cooking temperatures are reached. People associate residual pink color of hamburgers with undercooked product.

However, some properties of DFD beef can be advantageous. The high pH confers a greater water-holding capacity and bind strength of meat (Field et al., 1984), which significantly increase yield. Beef roasts from DFD meat are softer, more tender, and juicier than from normal beef (Hawrysh et al., 1985). Hawrysh et al. (1985) indicated that eating quality of DFD roasts is acceptable and similar to normal beef.

MEAT PIGMENTS

Myoglobin is the primary meat pigment. Myoglobin (Mb) is a monomeric oxygen-binding heme protein that is present in skeletal muscles of most vertebrate species. Structure and functions of heme proteins differ among species (Tsukahara, 1989; Lanari and

Cassens, 1991; Bembers and Satterlee, 1975). Myoglobin concentration is affected by species and muscle anatomical location. Postslaughter treatment of the carcass has a significant effect on the relative concentrations of myoglobin and hemoglobin. Myoglobin concentrations from 1.99 (*semitendinosus* muscle) to 3.64 mg/g (*biceps femoris* muscle) are reported for different beef muscles (Rickansburd and Henrickson, 1967). Hemoglobin (blood pigment) values, expressed as a percentage of total pigments, ranged from 20% (*longissimus dorsi* muscle) to 37.7% (*psoas major* muscle). Total pigment values for beef ranged from 3.02-6.54 mg/g (Krzywicki, 1982). When heme iron is in the ferrous (Mb) form, myoglobin can bind molecular oxygen reversibly to transfer oxygen from blood capillaries to mitochondria in red muscle. However, oxymyoglobin (OxyMb) is easily oxidized in vitro to MetMb, which cannot be oxygenated and is therefore physiologically inactive (Livingston and Brown, 1981). Autoxidation of Mb and hemoglobin to the ferric (MetMb) form is an intensely studied reaction. A summary reaction is shown below (Cornforth, 1994):

$$Mb + O_2 + 2H^+ + e^- \rightarrow MetMb + H_2O_2 \qquad (1)$$

Myoglobin heme iron donates one electron to oxygen. Various one-equivalent reductants provide the second electron (Al-Shaibani et al., 1977). The reaction rate depends on temperature, oxygen tension, pH, ionic strength, and concentrations of oxidants or reducing substances. Autoxidation, denaturation, and aggregation all may lead to the loss of the reversible oxygen-binding capacity and loss of the desirable color of fresh meat. Myofibrillar ATPase activity is positively related to glycolytic activities and negatively

related to oxidative activities (Talmant et al., 1986). Among the factors influencing the rate of autoxidation of MbO_2, the effect of pH has been widely investigated by several authors with a variety of mammalian and fish myoglobins (Livingston et al., 1986; Pan and Solberg, 1972). Shikama and Sugawara (1978) studied kinetics of autoxidation of bovine heart oxymyoglobin over the pH range of 4.8-12.6 in 0.1 M buffer at 25°C. The pH-profile for autoxidation rate is explained by an acid/base-catalyzed three-states model. The rate of autoxidation increases rapidly with increasing hydrogen ion concentration, and at pH 9 there appears the rate minimum followed by a small, but considerable increase at higher pH. The structural changes of bovine myoglobin occur as a result of pH change (Pan and Solberg, 1972). Some oxygen consumption rate of postrigor beef muscle is unaffected by the degree of comminution of the muscle (Bendall and Taylor, 1972). However, the respiration quotient in the postrigor state is 1.0 at low pH and falls to $\approx$ 0.5 on raising the pH to 7.2, due to mitochondria activity and initial production and subsequent oxidation of α-glycerol phosphate and pyruvate in equal amounts. The difference between species in the magnitude of the postrigor oxygen consumption rate is possibly due to differing mitochondrial contents (Lanari and Cassens, 1991). Ledward et al. (1977) found that oxidation of oxymyoglobin in minced beef during storage at 1°C is a first-order reaction. The rate constants for different muscles, although being pH dependent, are similar for all major beef muscles. The rate of reaction is about four to five times faster than that reported for oxymyoglobin in solution, indicating that catalysis occurs. The enzymatic MetMb reducing system is destroyed by mincing. Formation of MetMb in intact meat is far more variable and at least 10-fold slower

than in minced meat, in which the reducing system has been destroyed (Ledward et al., 1977; Ledward, 1972). The most significant factor affecting color stability of beef muscles appears to be their enzymatic activity, which determines the rate of myoglobin oxidation (Rennerre and Labas, 1987; O'Keeffe and Hood, 1982). Lanari and Cassens (1991) reported that muscles and breeds of lower color stability had the highest levels of oxygen consumption rate and mitochondrial metmyoglobin-reducing activity. There are no differences between metmyoglobin reductase activities measured in aerobic or anaerobic conditions (Echevarne et al., 1990). Reducing activity of bovine metmyoglobin reductase is maximal at a pH near 7.3 and at a temperature 37.5°C. Surface myoglobin accumulation, metmyoglobin reductase activity, and oxygen consumption rate are affected by muscle type, postmortem aging, and fabrication method (Madhavi and Carpenter, 1993). However, color stability of muscle is similar after grinding, which increases oxygen consumption. An enzymatic reducing system for fish metmyoglobin had an optimum pH of 7 and an optimum temperature of 25°C (Al-Shaibani et al., 1977). The geometry of the heme iron site changes upon reduction of metmyoglobin (Tsukahara, 1989). The rate of autoxidation decreased with increasing pH whilst the rate of reduction is believed to increase with increasing pH (Ledward, 1985). It would be expected that higher pH muscle would be more color stable. Yin and Faustman (1993) reported that OxyMb and phospholipid oxidations are interrelated. Both oxidations are enhanced with increased temperature (37 > 20 > 10 > 4°C) and decreased pH (5.6 > 6.4 > 7.2). The myoglobin of porcine muscle is more susceptible to autoxidation, heat, and acid denaturation, when compared to bovine and

ovine myoglobin (Bembers and Satterlee, 1975). They found that Mb extract from pale soft and exudative porcine muscle autoxidized at a rate approximately double that of an extract from normal muscle. Chow (1991) found a relationship between the stability against denaturation and autoxidation of fish myoglobin: the higher the stability of Mb against denaturation, the lower the autoxidation rate constant. Freezing and thawing of meat caused autoxidation of bluefin tuna myoglobin (MetMb, 15-40%) depending upon pH (Chow et al., 1987). Addition of ascorbic acid, antioxidants, and phosphates to raw ground beef can retard myoglobin oxidation (Greene et al., 1971; Govindarajan et al., 1977; Manu-Tawiah et al., 1991). Spoilage bacteria have been identified as one of the agents responsible for accelerating the oxidation of OxyMb to MetMb. However, meat color is not a reliable indicator of bacterial load (Faustman et al., 1990). There is no significant growth of psychrotrophic microorganisms in ground beef during 1 week of storage at 2°C (Manu-Tawiah et al., 1991).

MICROBIAL SAFETY OF COOKED HAMBURGERS

Over 1.36 million megatons of domestically or imported ground beef are consumed annually in the United States (Hague et al., 1994). For the last decade, hundreds of foodborne illness cases from uncooked beef hamburgers have been reported. *Escherichia coli* O157:H7 is responsible for the most of outbreaks. *E. coli* O157:H7 was first recognized as a foodborne pathogen in 1982 (Rilley et al., 1983; Wells et al., 1983) and is now recognized as an important cause of foodborne disease,

with outbreaks having been reported in the US, Japan, Canada, and the United Kingdom. Illnesses caused by *E. coli* O157:H7 can range from a self-limiting watery diarrhea to life-threatening symptoms such as severe bloody diarrhea, kidney failure, and blood clot in the brain. The O157:H7 serotype is the predominant cause of human infections but other serotypes have also been implicated. In comparison to *Salmonella*, numbers of cases appear to be low but they seem to be increasing. In mid-January 1993, *E. coli* O157:H7 caused serious illness in 600 people who ate contaminated hamburger at Jack-in-the-Box restaurants in Washington, Idaho, California, and Nevada (CDCP, 1993). In just the past few years, some strains of *E. coli* have emerged that can thrive in salted foods like sausage or acidic foods such as apple juice, and certain strains of *Salmonella* have developed the ability to resist food-processing temperatures that kill other organisms. By studying strains from different outbreaks, LeClerc et al. (1996) found that the frequency of mutators in the *E. coli* and *S. enterica* isolates is far higher (5% and 6%, respectively) than the normal 1% mutation rate. Foodborne illness is caused by verocytotoxin-producing *E. coli* (VTEC). VTECs produce one or more verocytotoxins that are similar to a toxin produced by *Shigella dysentenae* type 1. The pathogenicity of these strains is not fully understood, but it involves adhesion to and colonization of the intestinal tract and the production of powerful toxins that act on the colon. The infectious dose is not known, but it may be very low, i.e., fewer than 10 cells. Clinical symptoms range from mild diarrhea to severe bloody diarrhea (haemorrhagic colitis), and in some sufferers include hemolytic

uremic syndrome and kidney failure, which can be fatal. Cattle appear to be the main source of infection. Most cases are associated with the consumption of undercooked beef hamburgers and similar foods, or raw milk. However, other foods have also been implicated. The mechanism of transmission in the food chain is not fully understood but the contamination of meat from intestinal contents at slaughter is probably an important factor. For most foods, testing for *E. coli* is not an effective control strategy and the setting of end-product specifications is not appropriate (Mermelstein, 1993). For most food manufacturers, surveillance of raw and in-process materials, finished products, and the manufacturing environment should be based on needs identified by a Hazard Analysis Critical Control Point (HACCP) evaluation, and the end-product specification. Quality assurance programs in slaughterhouses should stress the need to minimize fecal contamination of carcasses and to chill meat rapidly. Elsewhere in the food industry, procedures to ensure that incoming food materials and ingredients are of good quality should be in place and adhered to. Screening of raw meats for *E. coli* O157:H7 is not an effective control mechanism because isolation rates from raw beef are low, and the organism has been found in the feces of a small proportion of healthy cattle, so currently it is unlikely that it can be eliminated at the source.

Simple and reliable methods suitable for routine *E. coli* O157:H7 detection in foods are not widely available. Similarly, because of the low contamination rate of *E. coli* of meat, routine screening specifically for this organism is unlikely to be worthwhile or successful. The methods used provide only a 20% reduction of

possibility of contamination with *E. coli* O157:H7. Contamination rates in fresh meat are less than 4%, so the chance of isolating the bacteria from a few samples of fresh meats is small (Doyle and Schoeni, 1987). The minimum pH for growth is thought to be pH 4.5, but some strains of the organism can survive in low pH products such as mayonnaise and yogurt, particularly in refrigerated storage for several weeks (Buchanan and Klawitter, 1992). Refrigeration below 5°C is thought to prevent growth of *E. coli* O157:H7 and is an important hygiene measure. However, any organisms present are likely to survive at these temperatures perhaps for several weeks (Doyle and Schoeni, 1984). Also, there is little agreement on the best method to use for routine testing. The widely used standard methods for detection and confirmations of *E. coli* are not appropriate as many VTEC strains grow poorly or not at all at 44°C (Buchanan and Klawitter, 1992; Doyle and Schoeni, 1984). Currently traditional isolation methods for foods involve enrichment in a selective broth followed by plating into sorbitol MacConkey agar with additives. This agar is only suitable for O157:H7 strains. Most but not all O157 VTEC strains do not ferment sorbitol. The composition of the enrichment broth and plating agar is important if VTEC is to be isolated from contaminated materials, and several groups are working on determining the optimum combination of selective agents. However, extensive research on isolation techniques is being carried out and various commercial kits have become available recently (Mermelstein, 1993). For *E. coli* O157 (but not all VTEC serotypes), commercial kits are available for isolation and identification (ELISA methods) and for confirmation of

suspect colonies (latex agglutination). An immunoassay kit is available for Shiga-like toxins and it is reported to detect several different VTEC strains. Recipes for several effective broths and agars have been published but there is no consensus yet on which is the best.

Animal and animal products remain the major reservoir of salmonellosis. Present technology in meat plants cannot guarantee a *Salmonella*-free product if the incoming animals are carriers. Salmonellosis remains one of the most common meat-borne diseases in the US, UK, and Canada. It is only realistic to assume that any meat and especially poultry is contaminated with *Salmonella* and should be handled as such. *Salmonella* problems associated with fresh meats include undercooking, cross-contamination, and inadequate cooling. Several outbreaks of salmonellosis from beef hamburgers served in fast-food restaurants have been reported (Fontaine et al., 1978). A major factor contributing to outbreaks from hamburgers is consumption of raw or uncooked products containing infective *Salmonella* levels.

Control of *E. coli* O157:H7 and salmonellosis illness in humans requires good slaughterhouse hygiene and heat treatment of raw meat. If feces from an animal that is *E. coli* O157:H7-positive contaminates the carcass, the contamination could be spread during the grinding process. *E. coli* O157:H7 and *Salmonella* are destroyed by heat. Adequate cooking of meat will protect consumers from infection from these sources. Hygienic food handling and good chilled storage conditions are essential and should ensure that other foods do not become contaminated. The organism is heat-sensitive

and should be destroyed by the same temperature that is recommended to eliminate *Salmonella* and *Listeria*. The food industry should provide cooking instructions with hamburgers to ensure that they are adequately cooked, that the meat juices run clear, and that there are no pink bits inside cooked products. In the United States, the Food and Drug Administration and the Department of Agriculture now recommend that ground beef products should be cooked to an internal end-point temperature of 71.1°C, or 68.3°C (with 16 sec holding) for food-service operations (USDA, 1993). The advice in the UK is that minced beef and minced beef products including beef hamburgers should be cooked to a minimum internal temperature of 70°C for 2 min or equivalent. Outbreaks may also be caused by cross-contamination of ready-to-eat foods from raw foods or dirty utensils. Person-to-person spread also occurs and has caused outbreaks in hospitals, day care centers, infant schools, and nursing homes. It has not been possible to pinpoint the source of infection in many sporadic cases and small outbreaks. Normal good manufacturing and catering practices should ensure that the chance of cross-contamination occurring is minimized. The measures needed to protect consumers from *E. coli* O157:H7 are the same as those needed to protect against *Salmonella, Campylobacter, Listeria,* and most other non-spore-forming foodborne pathogens.

The value of screening raw meat is being debated widely. Undoubtedly screening will detect some contaminated material and this can then be designated to a secure heat-treatment process. However, since screening can never detect all

contaminated lots, it is a poor control procedure. Adequate cooking of meat is the only sure way of eliminating the danger of *E. coli* O157:H7 and *Salmonella* infection from this source. The site of least heat penetration in cooking of beef hamburgers is the core, and bacteria located there may not be destroyed unless the cooking process is complete.

Heat is the most widely used agent in food preservation because of its reliability and economic feasibility. The major concern of thermal processing is attainment of commercial sterilization or pasteurization while retaining quality of product. The kinetics of bacterial death is relatively well understood and kinetic data are available for bacterial destruction in a number of microorganisms (Jay, 1986). The rates of destruction can be related to the number of viable organisms at the start of heating and the number of viable organisms that can safely be allowed to survive. This is done by combining the thermal death time data with the temperature-time history at the point in the product that heats the slowest, usually the geometric center. The standard reference temperature is generally selected as 121.1°C and the relative time required to sterilize any selected organism at 121.1°C is the F value of the organism. The quality of a thermal process is determined by the temperature and duration of cooking. The parameters of a suitable thermal process may be estimated based on the heat resistance of the target microorganism and knowledge of the temperature history during processing.

Thermal resistance of microorganisms can be described by the D-value (time required for one log reduction of microorganism concentration at a given temperature)

and z-value (temperature change required to change the thermal death time by a factor 10; *i.e.*, a one log reduction). Line et al. (1991) determined D- and z-values for *E. coli* O157:H7 in extra lean (2% fat) and regular ground beef (30% fat). D-values for regular ground beef exceeded those for extra lean ground beef at the temperatures tested. D-values for extra lean and regular ground beef at 51.7°C are 78.2 and 115.5 min, respectively, as enumerated on plate count agar (PCA) plus pyruvate. D-values at 57.2°C are 4.1 and 5.3 min, and at 62.8°C are 0.3 and 0.5 min for extra lean and regular beef. The z-values determined for extra lean and regular beef using PCA are 4.61 and 4.67°C, respectively. Heat resistance of *E. coli* O157:H7 is affected by meat product composition (Ahmed et al., 1995). Goodfellow and Brown (1978) reported *Salmonella* D-values of 61-62, 3.8-4.2, and 0.6-0.7 min in ground beef at 51.6, 57.2, and 62.7°C, respectively. The z-value of the *Salmonella* serotypes is 5.56°C.

Using the experimental measurements at the slowest heating point in the hamburger, the temperature-time graph can be plotted. This curve is then evaluated in terms of its effectiveness in destroying the organism of concern to the processor. Experimentally, it has been found that if the logarithm of D, the thermal death time (TDT), is plotted against temperature, a straight-line relationship is obtained (Lund, 1975). The following Eq. 2 describes a TDT curve:

$$\log(D/F) = (T^* - T)/z \qquad (2)$$

where D = TDT at temperature T (min); F = TDT at reference temperature T* (min); T = Temperature (°F); T* = Reference temperature (°F); and z = the °F temperature

change required to change the TDT by a factor of 10. Equation (2) can be transformed to:

$$D = F\ 10^{(T^*-T)/z} \qquad (3)$$

The fraction of the process to reach thermal death, dS, accomplished in time dD is given by: $(1/D_1)dD$, where D_1 is the TDT at temperature T_1, assuming that the destruction is additive. Therefore,

$$dS = (1/D_1)dD = (1/F)10^{-(T^*-T)/z}dD \qquad (4)$$

Effective sterilization or destruction of microorganisms is achieved when the thermal death time has been reached, then:

$$\int dS = 1 \qquad (5)$$

that is $$\int (1/F)\ 10^{-(T^*-T)/z}dD = 1$$

or $$\int 10^{-(T^*-T)/z}dD = F \qquad (6)$$

At the moment when the integral is equal to F, sterilization is said to have been achieved. This value of F must equal a standard value of F for the organism concerned. Equation (6) can be evaluated either graphically or by numerical integration (Amanie, 1993). In the latter case, the contribution towards F or a period of a process, D (min) at temperature T, is given by:

$$D \times 10^{-(T^*-T)/z}dD \qquad (7)$$

By breaking up the time-temperature curve into D_1 (min) at T_1, D_2 (min) at T_2, etc., the total F can be transformed to:

$$F = D_1 \times 10^{-(T^*-T)/z} + D_2 \times 10^{-(T^*-T)/z} + \ldots \quad (8)$$

Thus the factors F and z combined with the temperature-time curve can be used to evaluate the heating process.

The Food Marketing Institute and American Meat Institute, in cooperation with the national Live Stock and Meat Board, USDA, and FDA, published guidelines for both consumers and food-service industry regarding safe handling and preparation of ground beef (FMI-AMI, 1993a,b). For both consumers and food-service, these guidelines suggested that patties be cooked until no pink color remained in the center and juices are clear. However, especially with low-fat patties from dark-cutting beef, considerable pink color may be evident at the internal end-point temperatures of 71°C suggested for consumers. Also, under identical cooking conditions, considerable variation in color may occur. There are several theories as to the cause, such as: use of cow meat, elevated muscle pH, use of particular types of processing equipment, and lengthy freezing times (Berry, 1992, 1993, 1994; Berry and Abraham, 1993; Berry and Wergin, 1993). There are no data on temperature cooking profile and microbial safety of hard-to-cook hamburgers for the typical food-service double-side frying procedure.

HEAT TRANSFER IN GROUND BEEF

Simultaneous heat and moisture transfer is a very complex process. Therefore, in many attempts made to solve the problem of conduction heat transfer, the two are dealt with separately, and sometimes moisture transfer is neglected completely from

heat transfer. During frying of beef hamburger on an electric grill, heat is transferred by conduction, convection, and radiation. There are many factors influencing heat transfer in beef hamburgers (Dagerskog, 1979; Rhee and Drew, 1977; Irmiter et al., 1967), including thickness, porosity of meat and compaction, fat content, initial temperature of hamburger (frozen or thawed), cooking method, and cooking temperature. Internal heating mechanisms depend from thawing of frozen water and melting of fat, evaporation of water at 100°C (phase changes), denaturation of protein at 57-75°C, and shrinkage and juice release at 60-70°C. Ground beef is a non-homogeneous product with the following typical composition by weight (Lawrie, 1968): water 60-75%, protein 18-20%, fat 3-30%, carbohydrates 1.2%, and inorganic salts 0.7%. Calculation of heat transfer in meat products requires identification of thermal properties, geometry of the food, and thermal processing conditions (Dickerson and Read, 1968). The thermal and physical properties relevant to the study of heat transfer characteristics are 1) thermal conductivity, 2) specific heat, 3) density, and 4) the heat transfer coefficient between surrounding air and the product surface. The early contributors in this area include Qashou et al. (1970) and Irmiter et al. (1967), who studied heat penetration rates in ground beef in relation to fat content.

There are several reports in the literature concerning measurements of thermal conductivity of beef. The functional relationships for various parameters of conduction heating can be derived using appropriate theoretical formulas. To predict the thermal conductivity of cooked beef, a model based on composition and temperature is

developed using the measured cooking reheating and recooling data (Baghe-Khandan and Okos, 1981). The thermal conductivity of beef hamburgers at 30-120°C in the cooking temperature range can be assumed to depend linearly on composition as shown in Eq. 9:

$$K = K_w X_w + K_f X_f + K_p X_p \qquad (9)$$

where K = Thermal conductivity (W/m°C); X = Fraction of composition; *Subscripts:* w = Water, f = Fat, and p = Protein. Models of the pure components are given in Eq.10 as a linear function of temperature and are determined from a standard optimization subroutine with the objective function as the minimization of the standard error:

$$\begin{aligned} K_w &= 5.94x10^{-1} + 9.57x10^{-4}T \\ K_f &= 1.79x10^{-1} - 2.23x10^{-4}T \\ K_p &= 1.72x10^{-1} + 2.81x10^{-4}T \end{aligned} \qquad (10)$$

where T = Temperature of the product in °C. Standard error and standard percent error for ground beef were 0.0339 and 7.62%, respectively. Low values of standard error and standard percent error suggest accurate prediction of thermal conductivity in ground beef. Authors found that the thermal conductivity of ground beef increased from 0.48 W/m°C at 30°C to 0.49 W/m°C at 69°C.

Sweat et al. (1973) developed thermal conductivity equations for dark and white meats. Thermal conductivity varied linearly above freezing (Eq. 11), but a second-order polynomial gave a better description of the data for temperature below freezing (Eq. 12).

$$K = 0.481 + 0.000865T \quad \text{(dark meat, 0-20°C)} \qquad (11)$$

$$K = 1.14 - 0.0146T - 0.986x10^{-4}T^2 \quad \text{(dark meat, -75 to -10°C)} \qquad (12)$$

where K = Thermal conductivity in W/m°C and T = Temperature in °C. Accuracy of the thermal conductivity values predicted by the above equations was estimated to be ± 5% of the value predicted for temperatures above freezing and ± 10% for temperatures below freezing. There is an abrupt change in thermal conductivity at the freezing zone due to freezing of water in the meat products (Lentz, 1961; Hill et al., 1967). This is expected since the thermal conductivity of ice is four times as great as that of water at 0°C.

Dagerskog (1979) listed the following relations (Eq. 13) for meat patties as a function of temperature (T), water (W), and fat (F) content:

$$\begin{aligned} K &= 0.5 - 0.92F + 0.0024T \\ \rho &= 1300 - 300W - 400F - (0.4W + 0.7F)xT \\ C_p &= 1600 + 2600W + 15TxF \end{aligned} \qquad (13)$$

where K = Thermal conductivity (W/m°C), ρ = Density (kg/m^3), C_p = Specific heat (J/kg°C).

McProud and Lund (1983) conducted experiments on uncooked and cooked beef loaves heated to 60°C with 72.1% and 66.2% moisture, and 17.6% and 13.0% fat content, respectively. They obtained thermal conductivity values of 0.40 W/m°C and 0.47 W/m°C and specific heat 3684 kJ/kg°C and 3809 kJ/kg°C, respectively.

Density is an important physical property for ground beef that contains a sizable amount of void space. Klein et al. (1969) reported density of uncooked bovine skeletal muscle to range from 1060-1070 kg/m^3 in agreement with density of 1070 kg/m^3 at 25°C reported by Jarvis (1971). Qashou et al. (1970) measured 928-974 kg/m^3 densities of ground beef, which is close to 1000 kg/m^3 density of uncooked ground beef, reported by McProud and Lund (1983).

The surface heat transfer coefficient is one important factor affecting heat transfer in meat products. Skjoldebrand (1980) determined the heat transfer coefficients for forced convection frying of minced meat loaves. The mean value of heat transfer coefficient at 150°C of hot air at 5 m/sec velocity is between 20-90 W/m^2°C. These values closely agree with heat transfer coefficients reported by Holtz and Skjoldebrand (1986). McProud and Lund (1983) calculated the heat transfer coefficient using data obtained during heating of beef loafs in forced convection gas oven at 163°C and 176°C to the desired end temperature of 74-77°C using the equation for unsteady-state heat transfer. The calculated heat transfer coefficient is 62 W/m^2°C from three trials for each on-premise food-service systems: convection, cook/chill, and cook/freeze.

The exact solution by the governing heat transfer equations is very difficult and sometimes impossible to obtain and therefore appropriate numerical solution techniques have to be sought. In doing this, certain assumptions are made (Singh, 1997): One-dimensional heat transfer and energy fluxes are orthogonal to the surface of the

hamburger; phase change in the core takes place due to pure conduction; initially hamburger is homogeneous and isothermal; thermophysical properties change only along the streamlines parallel to the energy fluxes; chemical and physical changes occurring in the product during heating are considered negligible; heating medium is at constant temperature; crust/core interface is sharp and of zero thickness; there is no shrinkage or mass transfer.

The use of the finite-element method (FEM) as a numerical procedure for solving differential equations and behavior simulation of a product's structure in food engineering has increased considerably (Puri and Anantheswaran, 1993). The method has been successfully used to model heating and cooling, freezing and thawing, and heat and mass transfer. The results of FEM can be used for optimization of food quality in terms of texture, nutrient retention, and microbial degradation during thermal processing. The theoretical basis of the method and its application to structural and solid mechanics problems has been discussed by DeBaerdemaeker et al. (1977). In finite-element analysis, the design is partitioned or subdivided into a series of elements that are connected by nodes. Material properties and element properties are specified to represent the physical properties of the model. Boundary conditions and applied loads are then defined to represent the operating environment for the design. This process is called finite-element modeling. The element type and number of nodes used in the modeling process are individually selected. Nodes are used to connect elements and are generated when the model is meshed. Proper simulation of reality depends on

the proper choice of element type and properties. The most popular two-dimensional element is the triangle. All elements are assumed to be connected at nodal points located along the boundaries. The element equations are obtained by minimizing a functional or a residual. The accuracy of the model results depends greatly on the proper choice of material properties, boundary conditions, and applied loads.

The theoretical aspect of the finite-element method of time-dependent heat conduction in axi-symmetric bodies is governed by:

$$\frac{\partial}{\partial r}\left(rk_{rr}\frac{\partial T}{\partial r}\right)+\frac{\partial}{\partial z}\left(rk_{zz}\frac{\partial T}{\partial z}\right)=r\rho C_{\rho}\frac{\partial T}{\partial t} \qquad (14)$$

where r and z are the cylindrical coordinate directions; k_{rr} and k_{zz} = The conductivities in the cylindrical coordinate directions; T = Temperature; ρ = Density of the material; Cp = Specific heat, and t = Time. Equation 14 is valid as long as there is no internal heat generation. The boundary conditions for Eq. 15 are either a prescribed temperature:

$$T = T_b \quad \text{on a surface } S_1 \qquad (15)$$

or convection gain or losses through the surface:

$$k_{rr}\frac{\partial T}{\partial r}\ell_r + k_{zz}\frac{\partial T}{\partial z}\ell_z + h(T-T_{\infty}) + q = 0 \quad \text{on a surface } S_2 \qquad (16)$$

where ℓ_r and ℓ_z are the direction cosines; h = Surface heat transfer coefficient; T_{∞} = Air temperature surrounding the body, and q = Boundary heat source. It is assumed that ρ, C_p, q, and h are rotationally symmetric. Also, S_1 and S_2 are mutually exclusive, with their sum equalling the total surface area. The Galerkin Residual

Method can be used to transform Eq. 16 into a finite-element form. The purpose of the finite-element method is to determine the solution to these equations across the entire system being analyzed. The simplest form of a basic equation is as follows (Amanie, 1993):

$$[K]\{d\} = \{A\} \qquad (17)$$

where $\{d\}$ = Degree of freedom vector, $\{A\}$ = Action vector, and $[K]$ = Matrix relating $\{d\}$ to $\{A\}$ (often called the stiffness or coefficient matrix). In general, $[K]$ and $\{A\}$ are known, and $\{d\}$ is initially unknown.

The actual form of the basic equation is determined by the type of analysis being performed. For instance, in a steady-state thermal analysis, the equation is:

$$[K]\{T\} = \{Q\} \qquad (18)$$

where $[K]$ = Thermal conductivity matrix, $\{T\}$ = Temperature vector, and $\{Q\}$ = Heat flow rate vector. In order to solve the basic equation across an entire system, the system must be represented (modeled) by discrete, interconnected pieces (elements). Once $[K]$ is determined for each element, all of the individual $[K]$ matrices are assembled to form the set of simultaneous equations. Solution of the simultaneous equations gives response values at every degree of freedom across the entire system.

Kumar et al. (1990) used FEM for numerical simulation of natural convection heating of canned thick viscous liquid food products. Results of research indicated that the natural convection moved the slowest heating point to the bottom center of

container. Ikediala et al. (1996) used FEM of heat transfer in meat patties during single-sided pan-frying.

HAMBURGERS

According to the USDA, ground beef may be formulated at up to 30% fat by weight (Federal Register, 1996). However, concerns on the part of consumers regarding diet and health are dictating changes in the marketing of meat products. The label name "extra lean" means 10% fat or less, while "lean" or "low fat" has been defined as no more than 22% fat. When beef patties have lower than 8% fat, a reduction of tenderness, juiciness, and flavor occurs (Berry, 1992). An internal end-point temperature of 71.1°C is suggested for consumers at home while 68.3°C (with 16 sec holding) is recommended for food-service operations (USDA, 1993; USDA-FSIS, 1993). The pink color of cooked ground beef is viewed as undercooked meat. Consumers often interpret red or pink color in beef products as an indication of undercooking. Medium or well-cooked and microbial safe beef products (cook temperature 71 and 75°C) made from dark-cutting beef are usually nonuniform pink colored inside. Undenatured meat pigments of dark-cutting beef are responsible for pink discoloration of such products. There is a time-temperature relationship for the change in color of muscle myoglobin in cooked beef from red to gray. A temperature of 145°F (63°C) causes a rapid change of color of the myoglobin from red to grey, whereas a temperature of 135°F (57°C) for prolonged periods (37 min) produces little color change of myoglobin (USDA, 1978; Trout, 1989). In a study by Schmidt and Trout

(1984), meat slurries of beef, pork, and turkey were pH-adjusted to 5.5, 6.0, and 6.5 and cooked to 145, 155, or 165°F. Even when cooked to the same internal temperature, the high pH beef, pork, and turkey was redder than the low pH meats. The authors suggested the reason for this phenomenon is that high pH meats protect myoglobin from being denatured. Trout (1989) agreed with these results and stated pH effect is the greatest at lower temperatures (55 and 62°C) where the percent denatured myoglobin is 3 to 14 times greater at pH 5.5 than at pH of 7.0. Denaturation of meat pigments could be increased by lowering the pH and increasing temperature of cooking of meat products. Metmyoglobin appears to be more resistant to heat denaturation than either myoglobin or oxymyoglobin especially at high pH (Janky and Froning, 1973). Warren (1994) stated that oxidized states of myoglobin produce premature brown color at 55°C in beef patties, whereas reducing conditions produce normal brown color at 71°C. Undenatured myoglobin and oxymyoglobin may be present in sufficient concentration to cause red color in meats cooked to 71°C, if pH greater than 6.0 (Trout, 1989; Schmidt and Trout, 1984). Hard-to-cook hamburgers, characterized by persistent internal red color during cooking, are associated with high pH raw meat, such as bull meat (Mendenhall, 1989).

Reducing conditions produce pink or red colors of meat pigments. In fresh meat, heme iron must be reduced (ferrous) in order for oxygen binding or bloom to occur. The standard oxidation-reduction potential (ORP) value (Eh, pH 7.0) for Mb/MetMb is +46 mV (Loach, 1970). In cooked meats, heme is exposed due to globin denaturation, and the heme iron is more rapidly oxidized than in fresh meats upon exposure to air. Thus, relatively

strong reducing conditions are needed for stable pink complexes to occur in cooked meat. The ORP at which pink color appeared upon titration with a strong reductant ranged from -321 to -511 mV (Cornforth et al., 1986). Galesloot and Kooy (1960) developed a method for measuring ORP in milk and cheese. They observed increasing ORP from Eh = -280 mV at pH = 7.5 to Eh = -70 mV at pH = 4.0 during souring of pasteurized milk at 20°C.

An important feature of beef hamburger is the ability of the protein matrix formed to effectively bind the meat particles together (Beilken et al., 1991). Effective bind is essential if the product is to retain its structural integrity during subsequent handling. Length of frozen storage influences properties of hamburgers. Increased frozen storage (0-22 wk) increase thaw and cook losses, and loss of surface color (Bhattacharya et al., 1988). A storage temperature of -18°C and below has minimal effects of ground beef patties (Berry, 1991).

It is known that high pH dark-cutting meat is hard to cook, with rare, or raw appearance even after cooking. Preliminary work showed that there are variations from mild to extreme dark-cutting with pH ranging from 6.0 to 7.0. There is little or no data available on cooking rates and physical properties of DFD patties, their acceptability, and especially the effect of fat on cooked meat properties.

BEEF PATTIES

As a result of the *Escherichia coli* O157:H7 outbreak associated with inadequate cooking of beef patties, various regulatory agencies and trade associations have issued

changes in regulations or made suggestions regarding the cooking of beef patties (FMI-AMI, 1993a,b). However, especially with low-fat patties from dark-cutting beef, considerable pink color may be evident at the internal end-point temperatures of 71°C suggested for consumers. Also, under identical cooking conditions, considerable variation in color may occur. Hunt et al. (1995) reported that myoglobin form is responsible for the internal color of well-done beef patties. Patties with oxymyoglobin and metmyoglobin are brown at 55°C and could easily be mistaken as being cooked enough to kill pathogenic bacteria. Deoxymyoglobin appeared red and under cooked at 55°C, and the color of these patties became progressively more brown at 65 and 75°C. If doneness of beef patties is based on internal cooked color alone, it could be valid only if the pigment at time of cooking is deoxymyoglobin. Mendenhall (1989) reported that red and pink color is observed inside cooked beef (bull meat) patties with a pH of 6.2, even though a final internal temperature of 71°C is achieved. For beef patties, the industry now refers to this phenomenon as "hard-to-cook hamburgers" (Cornforth, 1991).

FOOD ADDITIVES AS BROWNING AGENTS IN COOKED PATTIES

The nature of the myoglobin derivative and certain additives appeared to have an effect on the amount of pigment denatured by temperature (Janky and Froning, 1973). Glycogen and glucose are absent in dark-cutting beef. Addition of reducing sugars to dark-cutting beef patties during cooking could cause brown melanoidin pigments to develop and mask pink color of hard-to-cook patties. While nonenzymatic browning is a defect in some

products, it is a desirable attribute in others such as bakery products, snack foods, nuts, and roasted meats. Nonenzymatic brown discoloration, the Maillard reaction, results from the reaction of carbonyl and free amino groups, leading to the formation of brown melanoidin pigments. The minimum reactant requirements for Maillard browning are the presence of an amino-bearing compound, usually a protein, a reducing sugar, and some water. Reaction depends on such variables as temperature, pH, moisture content, presence or absence of metal ions, and effect of sugar structure. The optimum pH for the Maillard reaction is 7.8-9.2 (Ellis, 1959). The effect of sugar structure on extent of browning was noted by Maillard (Maillard, 1912). He found that the decrease in the extent to which common sugars brown is in the order D-xylose > L-arabinose > hexoses (D-galactose, D-mannose, D-glucose, and D-fructose) > disaccharides (maltose, lactose, and sucrose). The degree of pigment formation from a particular sugar is directly proportional to the amount of open-chain (free carbonyl) sugar in the equilibrium solution (Ellis, 1959). Fructose yielded the highest browning in fried potatoes followed by glucose (Marquez and Anon, 1986). Methods of detection include observation of the formation of a yellow or brown color monitored quantitatively at 420 or 490 nm. Products of Maillard reaction are effective inhibitors of lipid oxidation in ground pork patties (Bedinghaus and Ockerman, 1995). Addition of glucose to dark-cutting beef can also be advantageous by delaying microbial spoilage of meat (Newton and Gill, 1981). However, Ahn and Maurer (1989) reported that glucose (1%) increased heat stability of hemoglobin at 68°C, and that of cytochrome *c* at 85°C.

Salt significantly decreases the heat stability of Mb in the range of 68-85°C, but significantly increases the heat stability of cytochrome *c* (Ahn and Maurer, 1989). Increasing the sodium chloride concentrations (0.0-3.0%) increases the rate of metmyoglobin formation in ground beef (Trout, 1990). Mechanism of myoglobin (Mb) oxidation in raw meat by sodium chloride can be described as the reaction promoted by anions (Wallace et al., 1982).

$$\begin{aligned} &MbFe^{++}O_2 \rightarrow MbFe^{++} + O_2 \\ &MbFe^{++} + L^- + (H^+) \rightarrow MbFe^{++}L^-(H^+) \\ &MbFe^{++}L^- + (H^+) + O_2 \rightarrow MbFe^{+++}L^- + HO_2 \\ &HO_2 \rightarrow H^+ + O_2^- \quad (pK_a = 4.8) \end{aligned} \qquad (19)$$

where $L^- = Cl^-, N_3^-, CN^-$. Sodium chloride concentrations increase percent of myoglobin denaturation in beef, pork, and turkey muscle when heated to temperatures between 55 and 83°C (Trout, 1984, 1989).

Pink color of charbroiled beef patties could be masked by addition of caramel. Caramel colorant can be added to food. Although caramel colorants are exempt from formal FDA certification requirements, they are, nonetheless, monitored by the FDA to assure that their purity is in accord with specifications and that they are used in according with regulations (Title 21, Parts 74 and 82, of the *Code of Federal Regulations*). Sucrose is commonly used for making caramel colors and flavors (Fennema, 1985). It is heated in solution with acid or acidic ammonium salts to produce a variety of products used in food, candies, and beverages. Commercially, three types of caramel colors are produced. The most abundant is acid-fast caramel made with ammonium bisulfate catalyst to produce the

color for cola drinks. Another is a brewer's color for beef, made by heating a sucrose solution with ammonium ion, and the third is a baker's color produced by direct pyrolysis of sucrose to give a burnt sugar color. Heating D-glucose at about pH 4 produces polymeric or condensed-ring particles of 0.46-4.33 nm diameter. Caramel pigments contain hydroxyl groups of varying acidity, carbonyl, carboxyl, enolic, and phenolic hydroxyl groups.

Historically, meat processors have used lactic acid for acidification of fermented sausages. Sausage processors cannot use uncoated lactic acid as an acid for cured meat products because it coagulates raw meat proteins and ruptures meat emulsions. Encapsulation of lactic acid by a hydrogenated vegetable oil, which will melt at 58-62°C, allows the use of lactic acid for production of cooked meat products. By addition of encapsulated lactic acid to dark-cutting beef, normal pH 5.6 could be achieved. Denaturation of meat pigments could be increased by lowering the pH and increasing temperature of cooking of meat products (Janky and Froning, 1973).

Calcium peroxide has been used in dough conditioning formulations for many years (Tieckelmann and Steele, 1991). Calcium peroxide (CaO_2) is one of the most versatile and safest to store alternative inorganic oxidants or bleaching agents. The food-grade calcium peroxide meets the Food Chemical Code requirements for use in bakery applications (FCC, 1981). About 0.25-0.5% of CaO_2 is used in flour formulations. The byproducts of mixing calcium peroxide in water are lime (CaO), hydrated lime ($Ca[OH]_2$), and hydrogen peroxide (H_2O_2). Addition of CaO_2 to dark-cutting beef can oxidize and bleach the red color of myoglobin. Reaction of hydrogen peroxide with myoglobin forms peroxyl radicals,

which initiate lipid peroxidation in meat (Kelman et al., 1994). Many articles have been published about hydrogen peroxide and autoxidation of oxymyoglobin and lipid oxidation (Morey et al., 1973; Tajima and Shikama, 1987). According to the results and mechanistic considerations of Yusa and Shikama (1987), the overall reaction scheme of oxidation of OxyMb to MetMb with hydrogen peroxide can be written as:

$$\begin{aligned} &2MbO_2 \Leftrightarrow 2Mb(II) + 2O_2 \\ &Mb(II) + H_2O_2 \rightarrow ferryl - Mb(IV) + 2OH^- \\ &ferryl - Mb(IV) + Mb(II) \rightarrow 2MetMb(III) \\ &2OH^- + 2H^+ \rightarrow 2H_2O \\ &\overline{2MbO_2 + H_2O_2 + 2H^+ \rightarrow 2MetMb + O_2 + 2H_2O} \end{aligned} \qquad (20)$$

OxyMb is oxidized easily to MetMb with generation of the superoxide anion, which can be converted by the spontaneous dismutation into H_2O_2, this being also a potent oxidant of Mb and OxyMb (Wazawa et al., 1992; Tajima and Shikama, 1987). A scheme for formation of peroxyl radicals for metmyoglobin treated with hydrogen peroxide is (Kelman et al., 1994):

$$Mb(Fe^{III}) \xrightarrow{+H_2O_2} {}^{\bullet}Mb(Fe^{IV}=O) \xrightarrow{+O_2} {}^{\bullet}OOMb(Fe^{IV}=O) \qquad (21)$$

Beef lipid oxidation catalyzing activity of MetMb-H_2O_2 in the raw meat system may be due primarily to MetMb activated by H_2O_2 and secondly due to the nonheme iron released from MetMb by H_2O_2 (Rhee et al., 1987). In the cooked meat system, the Met Mb-H_2O_2 catalysis of lipid oxidation appeared to be due mainly to the nonheme iron released from MetMb by the H_2O_2 action. These results agree with those of Gatellier et al. (1995), who showed that lipid peroxidation (TBA-RS accumulation) is induced

by a H_2O_2-activated MetMb system. Lipid oxidation is greater in microsomal membranes from *psoas major* (color unstable) muscle than from *longissimus lumborum* (color stable) muscle during 8 days of storage. Morey et al. (1973) reported development of green pigment (ferrohemochromes) during reaction of tuna and sperm whale myoglobins in native and denatured states with H_2O_2. Hawrysh et al. (1985) indicated that eating quality of dark-cutting beef is acceptable and similar to normal beef.

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CHAPTER III

EFFECT OF FAT CONTENT AND MEAT PH ON TEMPERATURE PROFILE OF HAMBURGERS COOKED BY DOUBLE-SIDE FRYING ON AN ELECTRIC GRILL

ABSTRACT

Extra lean (3.3% fat) and lean (20.0% fat) hamburgers in three pH groups (≤ 6.0; 6.01-6.49; 6.50-6.92) were evaluated for cooking-temperature profile and physical properties after cooking to 71°C by double-side frying on an electric grill. A modified general method was used to evaluate microbial lethality of double-side frying of hamburgers. Neither cooking-temperature profile nor cooking time was affected by fat content or pH. Double-side frying to 71.1°C internal temperature was adequate for more than 6-log destruction of viable *E. coli* O157:H7 and *Salmonella* at the geometrical center of extra lean and lean hamburgers. The coldest spot during cooking was not at the geometrical center, but was on the circumferential surface as indicated by presence of a red ring of undenatured myoglobin and confirmed by the finite-element temperature distribution model. The highest cooked yield and lowest cooked density were observed for hamburgers with elevated pH. Both pH and fat level affected the deformation pattern during cooking. The results of penetration tests showed significantly higher bind strength with increasing pH of hamburgers.

INTRODUCTION

During the last decade, hundreds of cases of foodborne illnesses were reported from uncooked beef hamburgers. *Escherichia coli* O157:H7 was responsible for most of the outbreaks. *E. coli* O157:H7 was first recognized as a foodborne pathogen in 1982 (Rilley et al., 1983; Wells et al., 1983) and is now recognized as an important cause of foodborne disease, with outbreaks having been reported in the US, Japan, Canada, and the United Kingdom. In comparison with *Salmonella*, numbers of cases appear to be low, but increasing. The organism is heat-sensitive and can be destroyed by the same cooking temperatures that were recommended to eliminate *Salmonella* and *Listeria*. In the United States, the Food and Drug Administration and the Department of Agriculture now recommend that ground beef products should be cooked to an internal end-point temperature of 71.1°C, or 68.3°C (with 16 sec holding) for food-service operations (USDA, 1993). If temperature is not directly measured, it is recommended that hamburgers be cooked until the meat juices run clear and there is no pink internal color.

Consumer demands for leaner meat and more efficient production have stimulated interest in the production of beef from bulls, though they have been considered more likely to produce dark-cutting beef than steers. The dark-cutting term is derived from the dark, firm, and dry appearance of meat with a high ultimate pH. Mendenhall (1989) reported that red and pink color is observed inside cooked beef (bull meat) patties with a pH of 6.2, even though a final internal temperature of 71°C was

achieved. Warren (1994) stated that metmyoglobin causes premature brown color at 55°C in beef patties, whereas reducing conditions produce normal brown color at 71°C. Thus, it is apparent that under identical cooking conditions, considerable variation in color may occur.

The quality of a thermal process is determined by the temperature and duration of cooking. The parameters of a suitable thermal process may be estimated based on the heat resistance of the target microorganism and knowledge of the temperature history during processing. Thermal resistance of microorganisms can be described by the D-value and z-value. Product composition also affects lethality of heat to *E. coli* O157:H7. Line et al. (1991) determined D- and z-values for *E. coli* O157:H7 in extra lean (2% fat) and regular ground beef (30% fat). D-values for regular ground beef exceeded those for extra lean ground beef at the temperatures tested. D-values for extra lean and lean ground beef at 51.7°C were 78.2 and 115.5 min, respectively, as enumerated on plate count agar (PCA) plus pyruvate. D-values at 57.2°C were 4.1 and 5.3 min, and at 62.8°C were 0.3 and 0.5 min for extra lean and regular beef. The z-values determined for extra lean and regular beef using PCA were 4.61 and 4.67°C, respectively. Ahmed et al. (1995) also reported that higher fat levels in red meats and poultry resulted in higher D-values of *E. coli* O157:H7. Goodfellow and Brown (1978) reported *Salmonella* D-values of 61-62, 3.8-4.2, and 0.6-0.7 min in ground beef at 51.6, 57.2, and 62.7°C, respectively. The z-value of the *Salmonella* serotypes was 5.56°C. Adequate cooking of hamburgers is the only sure way of eliminating the

danger of *E. coli* O157:H7 and *Salmonella* infection from this source.

The use of the finite-element method (FEM) as a numerical procedure for solving differential equations and behavior simulation of a product's structure in food engineering has increased considerably (Puri and Anantheswaran, 1993). The method has been successfully used to model heating and cooling, freezing and thawing, and heat and mass transfer. The results of FEM can be used for optimization of food quality in terms of texture, nutrient retention, and microbial degradation during thermal processing. The theoretical basis of the method and its application to structural and solid mechanics problems has been discussed by DeBaerdemaeker et al. (1977). Ikediala et al. (1996) used FEM to describe heat transfer in meat patties during single-sided pan-frying.

Density is an important physical property for ground beef that typically contains a sizable amount of void space. Qashou et al. (1970) measured densities of 928-974 kg/m^3 for ground beef similar to 1000 kg/m^3 for uncooked ground beef, reported by McProud and Lund (1983).

There are no data on cooking-temperature profile of hamburgers from dark-cutting beef for the typical food-service double-side frying procedure. The specific objective of this work was to determine physical characteristics and the effect of fat content and meat pH on adequacy of cooking hamburgers to 71°C internal temperature by double-side frying on an electric grill.

MATERIALS AND METHODS

Preparation of beef hamburgers

Frozen USDA choice inside rounds of normal and dark-cutting beef (pH > 6.0) were purchased from a local meat production plant. The rounds were trimmed of external and seam fat. The meat pH, fat, and moisture were checked before being made into hamburgers. Meat was sorted into three pH groups: normal beef (pH ≤ 6.0), mild dark-cutting beef (pH = 6.01-6.49) and extreme dark-cutting beef (pH = 6.50-6.92). Meat was thawed at 3°C in a cooler, then ground through a 0.32 cm plate. Finely ground (0.32 cm plate) frozen beef fat was mixed with extra lean meat (about 4% fat) to adjust the fat level to 20%, then re-ground. Ground meat was spread in a thin layer on plastic trays and kept in a cooler for an hour to allow meat to bloom. Hamburgers with two fat levels and three pH groups were manually formed using a 4S 3/8 mold (Hollymatic Corp., Park Forest, IL) and separated by glassine paper. The meat temperature was maintained at about 5°C during grinding and forming. Wooden toothpicks (7 cm length) were inserted from the side to the geometric center of the hamburger (12 cm diameter). Toothpicks were easy removed from frozen hamburgers for insertion of thermocouple probes. Hamburgers were placed individually on aluminum trays and frozen in a blast freezer (-27°C) for an hour. After that hamburgers were tightly packaged in plastic bags and stored at -27 ± 5°C until needed (1-4 months).

Moisture, fat, and pH measurements

Moisture and fat were measured by the standard gravimetric procedure (AOAC, 1990a,b; Appendix A). An Orion pH meter model 420A (Orion Inc., Cambridge, MA) with Orion Triode pH electrode (Triode™, Model: 91-57BN) calibrated at pH 4.0 and 7.0 was used for pH measurement in raw ground beef. Readings of pH in raw ground beef were obtained by inserting the electrode in the center of a raw extra lean ground beef chub (3-5°C). Readings were taken after pH had stabilized in few minutes. Triplicate measurements were made on each sample.

Frying equipment

Hamburgers were fried using a Hotpoint electric grill, model HG4 (General Electric, Chicago Heights, IL; Fig. 1) under a ventilation hood at air velocity about 5 m/sec. The grill consisted of a steel griddle plate, 600 x 500 x 25 mm with two temperature control devices, each connected to the heating elements mounted below the plate. Two thermostats on the electric grill controlled the heat such that the surface temperature on each half of the grill could vary from 203-550°F (95-285°C).

Frying operation

Hamburgers were taken from the freezer and tempered in a home refrigerator (3°C) until meat temperature was about -2°C (about 1 hr). Frozen hamburgers (about -1°C precooking temperature) were fried on one side on an electric grill preheated to 165 ± 5°C until internal temperature reached 40°C in the geometrical center (about 2.5 min), then

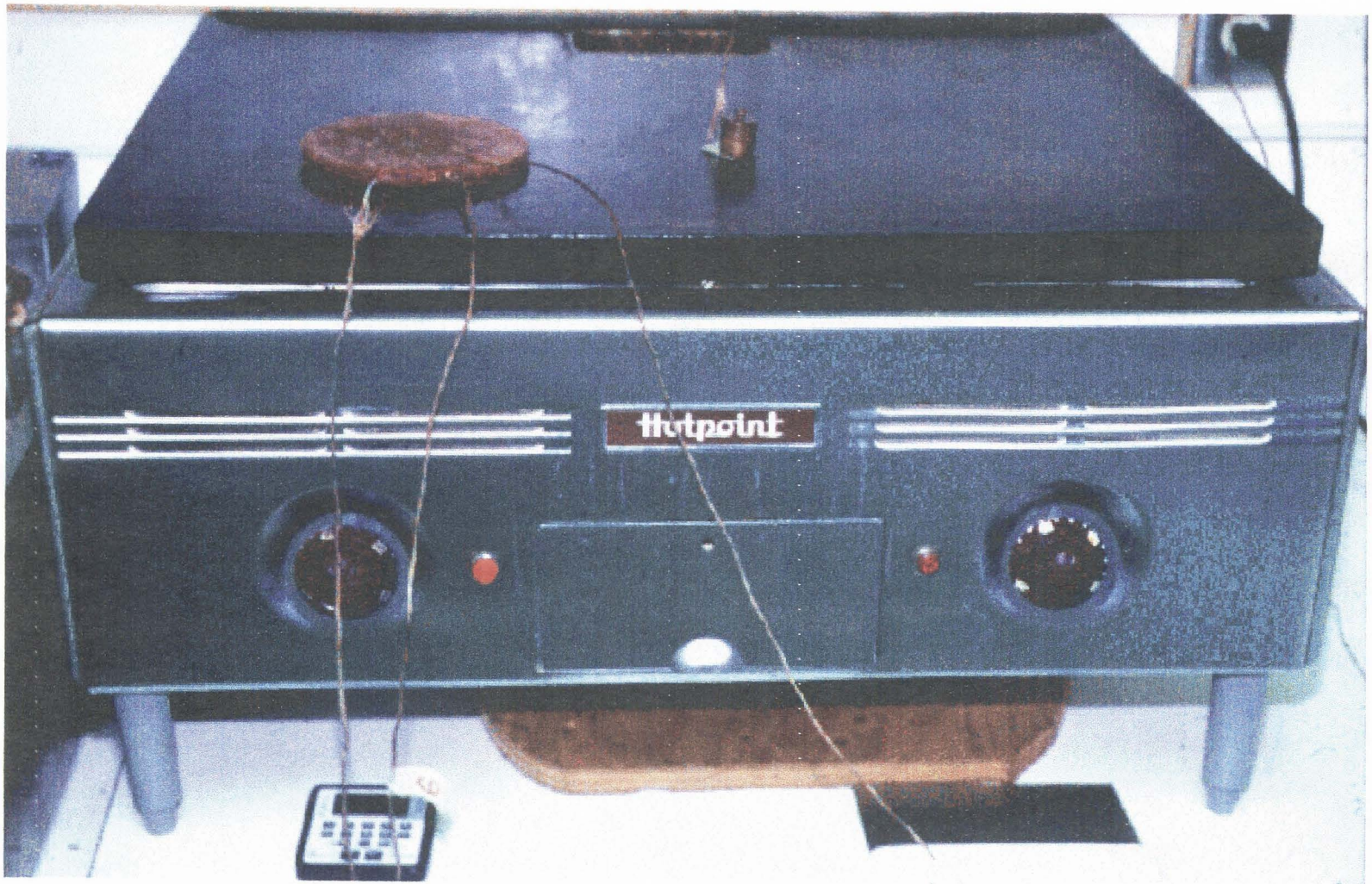


Fig. 1—Temperature measurements of hamburger by thermocouples during cooking.

flipped to the other side until the hamburger reached 71°C in the geometric center (about 4.5 min). Hamburgers were then removed and cooled to room temperature. Temperature of hamburgers during cooking was measured by inserting T-type copper-nickel thermocouples (calibrated at 0°C and 100°C) in the geometric center, and under the "skin" (1 mm) on the top and bottom side close to the geometric center axis. Temperature data were collected on an Easy Logger System (Omnidata International Inc., Logan, UT; Fig. 2) and transferred to an IBM PC through an RS 232 cable and use of the Terminal communication program on Windows 3.1 (Microsoft Corp., Redmond, WA).

Calculated lethality of hamburger double-side frying

By using the temperature measurements at the three points in extra lean and lean hamburgers, the temperature-time graphs were plotted. Temperature-time curves at the center of extra lean and lean hamburgers were pooled for all pHs, and used to calculate the cumulative lethality (F-value) of the cooking process compared to the accepted F-value for 6-log reduction of *E. coli* O157:H7 or *Salmonella*.

From the data of Ahmed et al. (1995), calculated F-values for 6-log destruction of *Escherichia coli* O157:H7 in extra lean and lean ground beef cooked to 71°C internal temperature were $F_{71.1°C}^{4.78°C} = 0.16$ sec and $F_{71.1°C}^{4.35°C} = 0.17$ sec, respectively, where superscripts are the respective z-values. Similarly, the F-value for *Salmonella* in ground beef, as calculated from the data of Goodfellow and Brown (1978), was $F_{71.1°C}^{5.56°C} = 0.12$ sec.

Fig. 2—Easy Logger System and measuring instruments kit.

Experimentally, it has been found that if the logarithm of D, the thermal death time (TDT), was plotted against temperature, a straight-line relationship was obtained (Lund, 1975). The following equation (22) describes a TDT curve:

$$\log(D/F) = (T^* - T)/z \qquad (22)$$

where D = TDT at temperature T (min); F = TDT at reference temperature T* (min); T = Temperature (°C); T* = Reference temperature (°C); and z = the °C temperature change required to change the TDT by a factor of 10. Equation (22) can be transformed to:

$$D = F\ 10^{(T^*-T)/z} \qquad (23)$$

The fraction of the process to reach thermal death, dS, accomplished in time interval dD was given by: $(1/D_1)dD$, where D_1 was the TDT at temperature T_1, assuming that the destruction was additive. Therefore,

$$dS = (1/D_1)dD = (1/F)10^{-(T^*-T)/z}dD \qquad (24)$$

Effective sterilization or destruction of microorganisms was achieved when the thermal death time has been reached, then:

$$\int dS = 1 \qquad (25)$$

so,
$$\int (1/F)\ 10^{-(T^*-T)/z}dD = 1$$

or
$$\int 10^{-(T^*-T)/z}dD = F \qquad (26)$$

At the moment when the integral is equal to F, sterilization is said to have been achieved. This value of F must equal a standard value of F for the organism concerned.

Equation (26) can be evaluated either graphically or by numerical integration (Amanie, 1993). In the latter case, the contribution towards F or a period of a process, D (min) at temperature T, was given by:

$$D \times 10^{-(T^*-T)/z} dD \qquad (27)$$

By breaking up the time-temperature curve into D_1 (min) at T_1, D_2 (min) at T_2, etc., the total F is given by:

$$F = D_1 \times 10^{-(T^*-T)/z} + D_2 \times 10^{-(T^*-T)/z} + \ldots \qquad (28)$$

Factors F and z combined with the temperature-time curve were used to evaluate the heating process.

Finite-element analysis of heat transfer

The finite-element analysis QuickField ™ Version 3.4 computer program (Tera Analysis Co., Tarzana, CA) was used to analyze steady-state heat transfer in extra lean and lean hamburgers. QuickField is a PC-oriented interactive program for electromagnetic, thermal, and stress analysis. Certain assumptions were made for heat transfer in hamburgers according Singh (1997): One-dimensional heat transfer and energy fluxes were orthogonal to the surface of the hamburger; phase change in the core takes place due to pure conduction; initially, hamburger was homogeneous and isothermal; thermophysical properties change only along the streamlines parallel to the energy fluxes; chemical and physical changes occurring in the product during heating were considered negligible; heating medium was at constant temperature; crust interface was sharp and of zero thickness; there was no shrinkage or mass transfer.

Experimental, theoretically calculated and literature values of heat transfer characteristics were used for finite-element analysis of heat distribution in cooking hamburgers. The functional relationships for various parameters of conduction heating can be derived using appropriate theoretical formulas. To predict the thermal conductivity of cooked beef, a model based on composition and temperature was developed using the measured cooking, reheating, and recooling data (Baghe-Khandan and Okos, 1981). The thermal conductivity of beef hamburgers at 30-120°C in the cooking-temperature range can be assumed to depend linearly on composition as shown in Eq. (29):

$$K = K_w X_w + K_f X_f + K_p X_p \qquad (29)$$

where K = Thermal conductivity (W/m°C); X = Fraction of composition; *Subscripts:* w = Water, f = Fat, and p = Protein. Models of the pure components were given in Eq. (30), a linear function of temperature, and were determined from a standard optimization subroutine with the objective function as the minimization of the standard error.

$$\begin{aligned} K_w &= 5.94x10^{-1} + 9.57x10^{-4}T \\ K_f &= 1.79x10^{-1} - 2.23x10^{-4}T \\ K_p &= 1.72x10^{-1} + 2.81x10^{-4}T \end{aligned} \qquad (30)$$

where T = Temperature of the product in °C. Low values of standard error (0.0339) and standard percent error (7.62%) suggest accurate prediction of thermal conductivity in ground beef. Thermal conductivity of extra lean and lean hamburgers was calculated

using overall mean values of proximate analysis data (Table 1), and using mean temperatures at flipping and end-point cooking time. Different thermal conductivity of extra lean and lean hamburgers, calculated by Eq. 30 (Table 2), is well correlated with thermal conductivity measured by Qashou et al. (1970) in ground beef at different percentages of beef fat.

The surface heat transfer coefficient is one important factor affecting heat transfer in meat products. McProud and Lund (1983) calculated the heat transfer coefficient using data obtained during heating of beef loafs in forced convection a gas oven at 163°C and 176°C to the desired end temperature of 74-77°C, using the equation for unsteady-state heat transfer. The calculated surface heat transfer coefficient was 62 $W/m^2°C$ from three trials for each on-premise food-service system: convection, cook/chill, and cook/freeze. Value of 62 $W/m^2°C$ as the surface heat transfer coefficient at air forced convection and 20°C air temperature was used to describe boundary condition at circumferential surface of hamburger during grill frying in a ventilation hood.

The calculated thermal conductivity of extra lean and lean hamburgers at flipping, and end-point cooking temperatures (Table 2) was used as a material property of meat in the finite-element analysis. Boundary conditions for bottom and top surfaces of hamburger were applied at prescribed temperatures according to experimental data (Fig. 3). Postprocessing results were presented as an isotherm color map of heat distribution in a meshed axi-symmetric model of hamburger within Z and R coordinate axes.

Table 1 — Proximate analysis of raw extra lean and lean hamburgers of pH = 5.80-6.73

Fat level	Raw meat pH	Moisture (%)	Fat (%)	Protein[1] (%)
Extra lean[2]	5.80	73.0 ± 1.1	3.3 ± 0.9	21.8 ± 1.2
Extra lean	6.29	73.4 ± 0.7	3.3 ± 0.9	21.4 ± 1.2
Extra lean	6.73	73.8 ± 0.8	3.3 ± 0.9	21.0 ± 1.1
Overall mean[3]		73.4 ± 0.9[a]	3.3 ± 0.9[b]	21.4 ± 1.2[a]
Lean	5.80	61.2 ± 1.2	19.8 ± 1.3	17.0 ± 2.1
Lean	6.29	60.6 ± 1.3	20.3 ± 1.0	17.1 ± 1.5
Lean	6.73	61.2 ± 1.0	20.0 ± 1.2	16.9 ± 1.7
Overall mean		61.0 ± 1.2[b]	20.0 ± 1.2[a]	17.0 ± 1.7[b]
Fisher's LSD		0.8	0.3	0.9

1 Percent protein was calculated by difference using typical beef composition for carbohydrates (1.2%) and inorganic salts (0.7%; Lawrie, 1968).

2 Values are means (n = 15) ± standard deviation.

3 Values are means (n = 45) ± standard deviation.

ab Overall means with at least one common superscript letter are significantly different ($p<0.05$; Fisher's least significant difference test; Tables 46-48, Appedix B).

Table 2 — Calculated thermal conductivity at the geometric center of hamburgers midway through cooking (when hamburgers were flipped) and at the end of cooking to 71°C internal temperature

Fat level	Thermal conductivity at middle-point time (W/m°C)[1]	Thermal conductivity at end-point time (W/m°C)[1]
Extra lean	0.510	0.533
Lean	0.453	0.470

[1] Thermal conductivity was calculated using Eq. 29. Overall composition and cooking temperature data of extra lean and lean hamburgers were from Table 1 and Table 3.

The model was validated by comparing predicted and experimental temperatures at the geometrical center of hamburger.

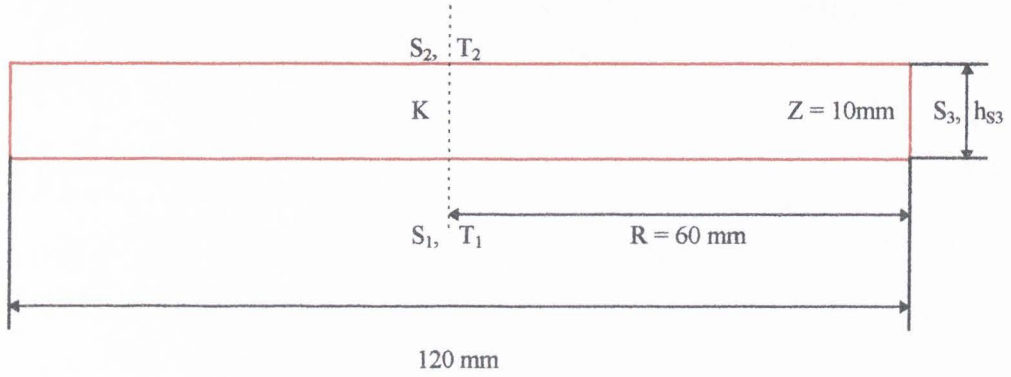
Physical measurements

Density of beef raw and cooked hamburgers (kg/m^3) was determined by a weight/volume method. Volume of raw and cooked hamburgers was calculated by using the formula for volume (V):

$$V = \pi R^2 h \qquad (31)$$

where $\pi = 3.1416$, R = radius of hamburger (m), h = height of hamburger (m). Weight of raw and cooked hamburger (kg) was measured by top-loading balance (Sartorius T 6, Baxter Scientific Products, SLC, UT).

(a) S_1, S_2, S_3 - bottom, top and circumferential surfaces, respectively; T_1, T_2 - prescribed temperature under the crust of surface S_1 and S_2 ; K - meat thermal conductivity; h_{S3} - heat transfer coefficient of surface S_3.



(b) Partition of axi-symmetric segment of hamburger,

→ - position of thermocouples in the geometric center axis

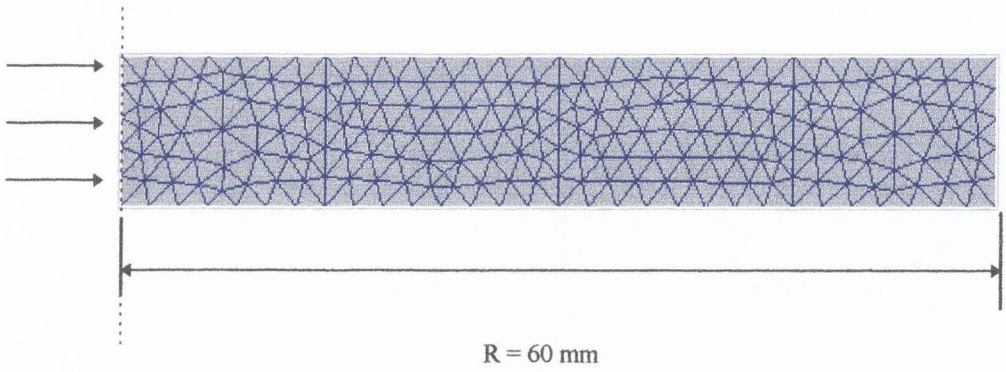


Fig. 3—Schematic view of hamburger: (a) cross section of hamburger; (b) axi-symmetric segment partition into 396 elements (triangulars) and 243 nodes (intersection points).

Percentage change in hamburger thickness was ascertained as follows:

$$\Delta \text{ patty thickness } (\%) = \left(\frac{\text{Raw patty thickness} - \text{Cooked patty thickness}}{\text{Raw patty thickness}} \right) \text{x } 100 \quad (32)$$

Percentage change in hamburger diameter was determined as follows:

$$\Delta \text{ patty diameter } (\%) = \left(\frac{\text{Raw patty diameter} - \text{Cooked patty diameter}}{\text{Raw patty diameter}} \right) \text{x } 100 \quad (33)$$

Ten hamburgers were used for raw (at 5°C) and cooked hamburger (at 20°C) thickness and diameter measurements. Two measurements were taken per hamburger.

Cooked yield was determined on 10 hamburgers by calculating weight differences for hamburgers before and after cooking as follows:

$$\text{Cooked Yield } (\%) = \frac{\text{Cooked weight}}{\text{Raw weight}} \text{ x } 100 \quad (34)$$

Penetration measurements

Penetration measurements were made using the penetrometer described by Dobson et al. (1993). The cooked hamburgers (at 20°C) were mounted on a plexiglass cylinder, similar to that described by Field et al. (1984). The hamburgers were held in place by tapered needles, 0.4 cm apart and protruding 1.25 cm above the surface of the cylinder. The circle formed by the needles was 9 cm in diameter. The cylinder + cooked beef hamburger was placed on a top-loading balance with digital readout and 1-g readability (Sartorius PT 6, 6000 g capacity, Baxter Scientific Products, Salt Lake City, UT), centered under the penetrometer rod, and tared to zero. The rod was advanced at maximum speed

(2 cm/min) and load (g) was recorded in 1-sec intervals until the polished steel ball (1.9 cm diameter) on the end of the rod penetrated through the hamburger (Fig. 4). The balance was connected to an IBM-compatible computer by a standard RS 232 cable. A specially developed Quick-Basic program was used to collect data and specify the time interval between recorded values.

Experimental design and statistical analysis

Data were analyzed as a completely randomized design. Treatments were arranged as a 3 x 2 factorial in a split-plot design, where three pH groups — normal beef (pH ≤ 6.0), mild dark-cutting beef (pH = 6.01-6.49), and extreme dark-cutting beef (pH = 6.50-6.92) — were the whole plot factors with two levels of fat — extra lean (target ≤ 5% fat) and lean (target 20% fat) — as the subplot factor. Five replications (sources) were performed per treatment and two samples were used for each experimental unit.

Experimental data were analyzed using the Statistical Analysis System program (SAS, 1988). ANOVA and multiple comparison Fisher's LSD ($p<0.05$) was used for statistical analysis of composition, cooking temperatures and physical parameters of hamburgers (Tables 46-66, Appendix B).

RESULTS AND DISCUSSION

Cooking temperature profile

Temperature-time curves for the top, middle, and bottom of hamburgers at the geometric center axis were obtained for 165°C grill frying of extra lean and lean

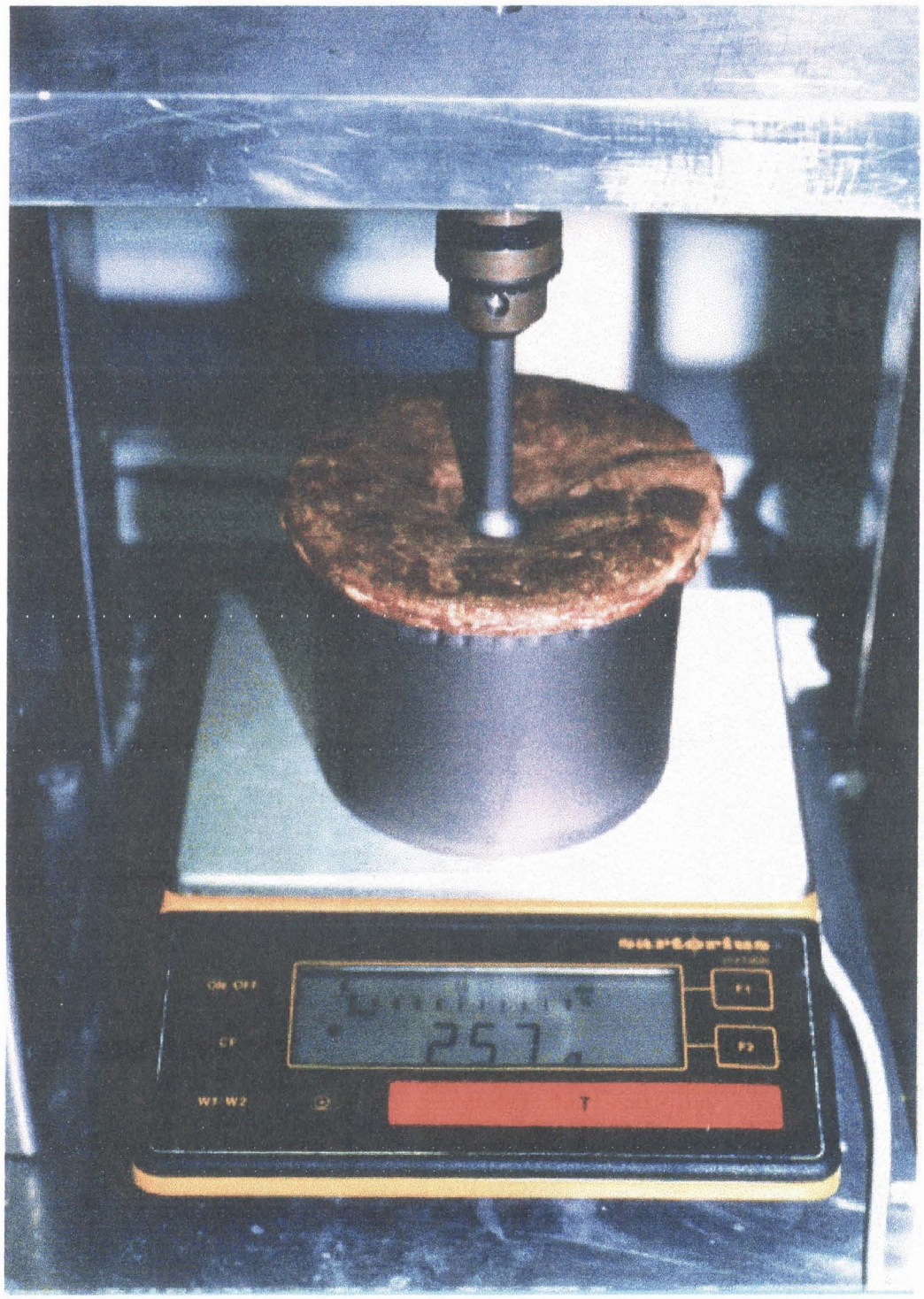


Fig. 4–Hamburger penetration test.

hamburgers (Figs. 5, 6). There was no difference in cooking curves patterns among pH groups or fat levels. Starting, flipping and end cooking temperatures were also not significantly different among pH groups or fat levels (Table 3; Tables 49-57, Appendix B). Starting middle temperature was -1.6 ± 0.6°C for all frozen hamburgers with slight variation on the top and bottom surfaces. The temperature of the bottom side rose sharply to 77-79 ± 5°C during early stages of heating, but after flipping gradually decreased to 49 ± 5°C at the end point of frying. The midpoint temperature gradually rose to 71°C at the end point of cooking. The top side did not show much response to heating during the first 120 sec, but reached 80 ± 3°C at 120 sec after flipping. After reaching 71°C internal temperature, the burgers were removed from the grill, but the midpoint temperature continued to rise in average to 72.2 ± 1.2°C (Table 3). The center temperature was apparently unaffected by the flipping. The entire hamburger had the same temperature of 60°C at 210-220 sec after cooking started, as shown by the intersection point of the three temperature-time curves. At the end of cooking, the bottom side temperature was almost the same as the center temperature, especially during the cooling step for 60 sec after 71°C was reached. Ikediala et al. (1996) reported a similar cooking-temperature profile for single-sided pan-frying of meat patties. Cooking time (230 ± 26 sec) was not significantly different between pH groups or fat level of hamburgers, probably because of high temperature-time deviations within groups (Table 3; Table 62, Appendix B). In general, however, frying of lean hamburgers took a few seconds longer than extra lean hamburgers, that possibly due to

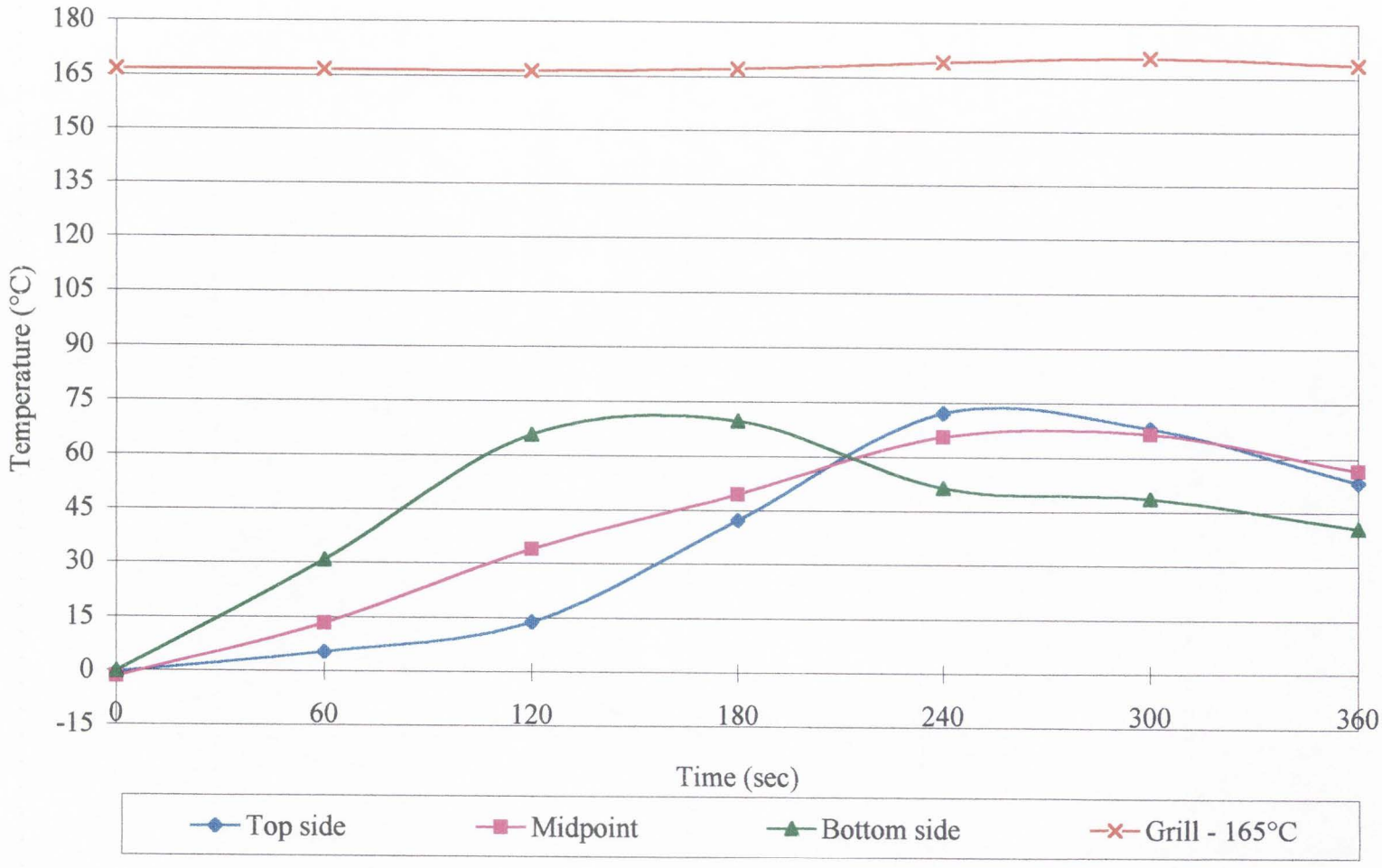


Fig. 5 –Temperature-cooking profile of extra lean hamburgers pooled for all pHs (n=30).
Hamburgers were removed from the grill when internal temperature reached 71°C.

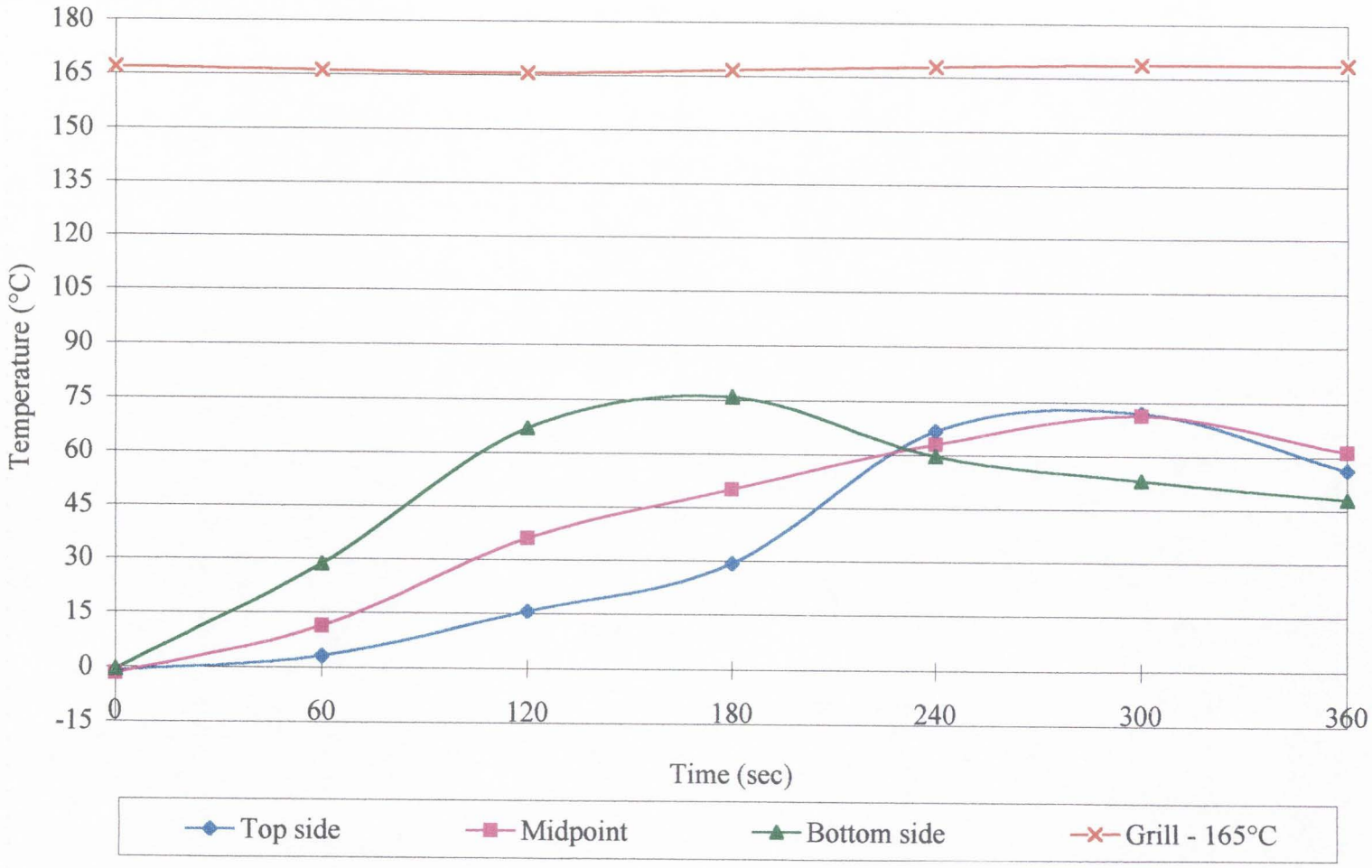


Fig. 6—Temperature-cooking profile of lean hamburgers pooled for all pHs (n=30).
Hamburgers were removed from the grill when internal temperature reached 71°C.

Table 3 — Mean hamburger temperature at three locations during cooking to 71°C internal end-point temperature

		Starting temperature (C)			Temperature when flipped (C)			End cooking temperature (C)		
Fat level	Raw meat pH	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
Extra lean[1]	5.80	-1.2±0.9	-1.5±0.7	-0.2±1.5	14.6±5.5	40.4±3.5	80.9±3.3	50.6±3.9	72.5±1.1	80.2±2.5
Extra lean	6.29	-1.0±1.1	-1.8±0.3	-0.7±3.2	12.2±5.2	43.9±3.4	76.7±4.2	51.3±5.1	72.2±0.6	79.8±2.9
Extra lean	6.73	-1.0±1.0	-1.6±0.6	0.1±1.0	8.8±5.3	42.8±2.5	75.6±3.1	46.2±4.3	72.5±1.6	79.1±3.2
Overall mean		-1.1±1.0	-1.6±0.5	-0.3±2.1	11.9±5.7	42.4±3.4	77.7±4.1	49.4±4.9	72.4±1.2	79.7±2.8
Lean	5.80	-0.4±1.1	-1.4±0.5	1.1±1.6	15.8±6.1	46.1±5.9	79.4±5.2	49.9±6.0	70.8±.08	80.2±3.4
Lean	6.29	-0.1±2.3	-2.0±0.8	0.2±2.1	12.5±5.5	42.4±2.6	78.9±5.6	46.9±5.7	72.2±1.5	80.4±5.1
Lean	6.73	-0.9±0.8	-1.7±0.6	0.6±1.6	13.4±6.8	44.7±2.9	78.8±4.9	50.5±5.8	72.1±1.3	81.5±4.6
Overall mean		-0.4±1.6	-1.7±0.7	0.6±1.7	13.9±6.1	44.4±4.2	79.0±5.1	49.1±5.8	72.0±1.2	80.7±4.3

[1] Values are means (n = 10 and n = 30 for overall mean) ± standard deviation.

the lowering of thermal conductivity with increasing of fat level (Table 2). Significant differences in initial weight, density and textural properties of extra lean versus lean hamburgers should be taken in to consideration. A wide variability of beef patty cooking times at constant internal end temperature, and variable internal end temperatures at constant cooking time have been reported. Cooking times with different cooking conditions and patty sizes vary from 3.5 to 6.5 min to reach 71°C temperature for thawed patties of different fat levels (Berry, 1993; Hague et al., 1994; Troutt et al., 1992a,b). Berry (1994) stated that frozen extra lean patties (4% fat) had 10 sec longer cooking time of 4:40 min than lean (20% fat) patties cooked on a grill at 160°C to ≥ 66.1°C for 60 sec. The explanation of cooking time difference between frozen beef patties with different fat content is a controversial issue. First, the thermal conductivity of ice is four times greater than water at 0°C (Lentz, 1961; Hill et al., 1967) and second, thermal conductivity of beef fat is lower than water (Lentz, 1961; Qashou et al., 1970). Therefore, as a general rule, with increasing percentage of water, the thermal conductivity of beef should increase and cooking time should decrease. However, it is not that simple, because meat has nonisotropic thermal properties that depend on cooking temperature and direction of heat flow with respect to meat fiber direction (DeBaerdemaeker et al., 1977). Difference in cooking time between extra lean and lean beef patties could better be explained by the difference in textural properties, density of ground beef, and mass loss than by thermal properties. The addition of beef fat trim and incorporation of air in meat by grinding make beef patties

less dense and more porous, and destroy the meat fiber network. Texture of beef patties may have greater effect on cooking time than difference in meat composition and thermal properties. If ground beef has less density and is more porous, it may take less time to cook the smaller mass. Hamburgers from dark-cutting meat had significantly lower raw and cooked density than normal pH hamburgers (Table 4; Table 59, Appedix B). Therefore, cooking time was slightly shorter for dark-cutting hamburgers (225 ± 25 sec) than for normal pH hamburgers (246 ± 27 sec). Fat particles were small and adequately distributed in meat mass. However, dark-cutting hamburgers had very sticky raw texture, that resulted in higher bind strength of cooked hamburgers (Table 4). These textural characteristics of ground dark-cutting beef may change the cooking time pattern of hamburgers, compared with literature data. Berry et al. (1996) reported that the temperature differences within patties cooked a constant time to achieve 71.1°C may range above 8°C. The cooking time was very short and temperature variations within product were too high to find critical difference in cooking time pattern of extra lean and lean hamburgers. Nevertheless, in this study cooking-temperature profile was similar for extra lean and lean hamburgers without any differences between pH groups. The cooking time pattern of extra lean and lean hamburgers was different from that reported in scientific literature.

Table 4 — Effect of pH on physical characteristics of hamburgers cooked to 71°C internal temperature

Raw meat pH	Initial weight (g)	Initial density (kg/m^3)	Cooked weight (g)	Cooked density (kg/m^3)	Cooking time (sec)	Yield (%)	Diameter shrinkage (%)	Thickness expansion (%)	Penetration load (g)
5.80	123.9 ± 4.7^{a}	1095 ± 42^{a}	107.7±7.3	1076 ± 85^{a}	246±27	86.8 ± 4.0^{b}	7.5±1.8	4 ± 7^{b}	523 ± 75^{c}
6.29	116.4 ± 4.6^{b}	1029 ± 41^{b}	107.9±4.9	1021 ± 52^{b}	222±26	92.6 ± 2.0^{a}	6.0±1.7	6 ± 6^{ab}	687 ± 120^{b}
6.73	117.9 ± 4.5^{b}	1042 ± 33^{b}	110.4±5.6	1006 ± 73^{b}	228±24	93.6 ± 2.4^{a}	6.5±2.3	11 ± 7^{a}	958 ± 159^{a}
Fisher's LSD	4.8	42.7	5.4	47	29.7	3.1	2.0	6	128

[1] Values are means (n = 20) ± standard deviation.

[abc] Means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Calculation of cumulative process lethality

By using the temperature cooking profile data at the geometrical center for extra lean and lean hamburgers pooled by all pHs shown in Fig. 7 and Fig. 8, the thermal process was evaluated. The thermal center should receive a heat treatment sufficient for 6-log destruction of viable *E. coli* O157:H7 and *Salmonella*. To determine if the heating process was adequate, the cumulative process F-values were calculated according to Eq. 28 and compared with the F-values calculated from the scientific literature.

Approximate stepped temperature increments were drawn on the temperature-time curves as shown in Fig. 7 and Fig. 8, giving the equivalent holding times and temperatures as shown in Table 5 through Table 7 for the given microorganism and product. The corresponding F-values were calculated for each temperature step. The results show that the cumulative F-value for the frying process was 6.12 and 9.88 sec, which is much higher than the values calculated from the literature (Ahmed et al., 1995) or USDA requirements (USDA, 1993) for 6-log destruction of *E. coli* O157:H7 in extra lean and lean hamburgers, respectively. The cumulative F-value for destruction of *Salmonella* was 8.76 sec in extra lean hamburgers, which was also much greater than the minimum F-value of 0.12 sec needed for 6-log *Salmonella* destruction (Table 7). In conclusion, the double-side frying of hamburgers to 71.1°C internal temperature is more than adequate for 6-log destruction of viable *E. coli* O157:H7 and *Salmonella*.

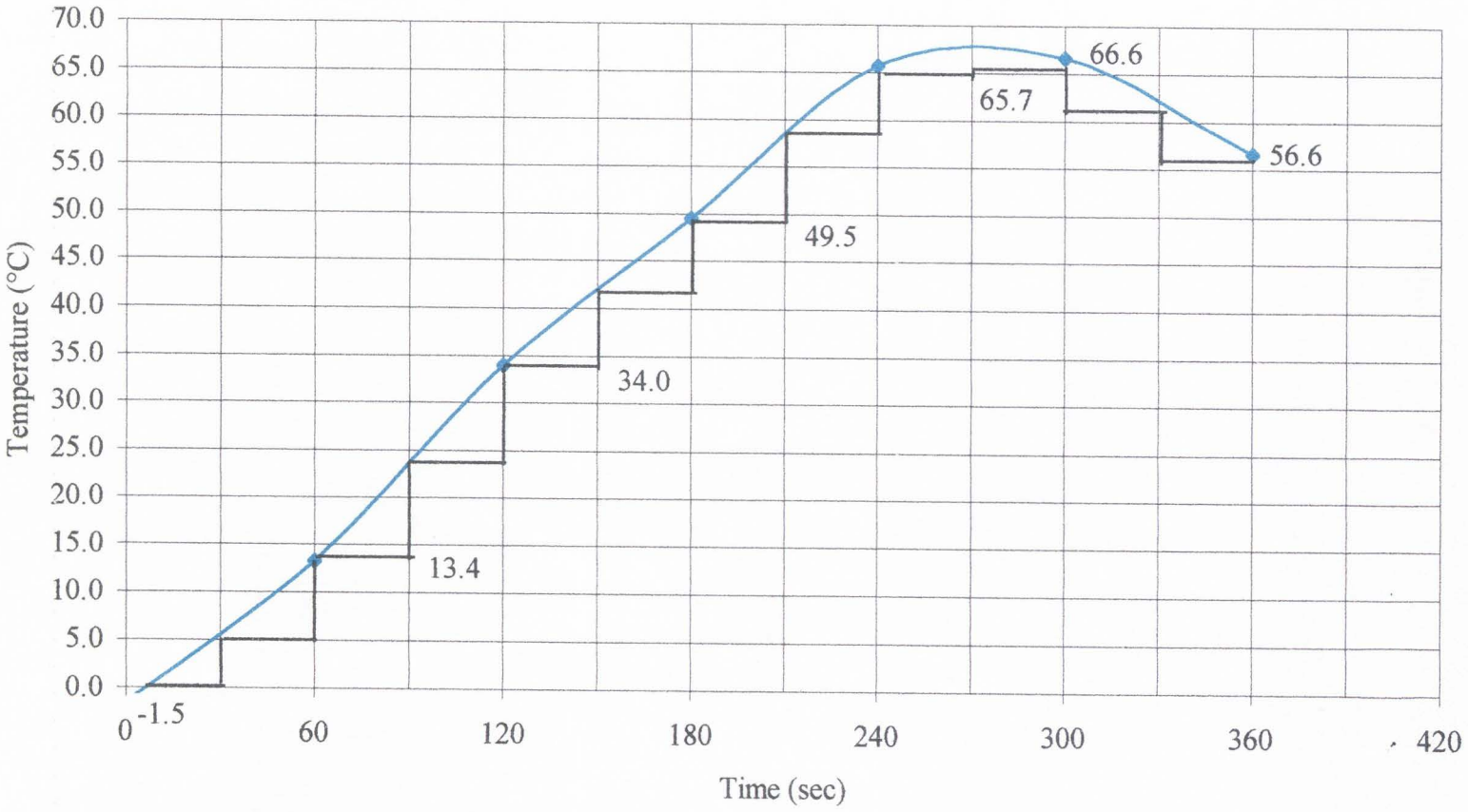


Fig. 7 -- Internal temperature at 30-sec intervals during cooking of extra lean hamburgers. (Grill temperature - 165°C; pooled for all pHs; n=30). Hamburgers were removed from the grill when internal temperature reached 71°C.

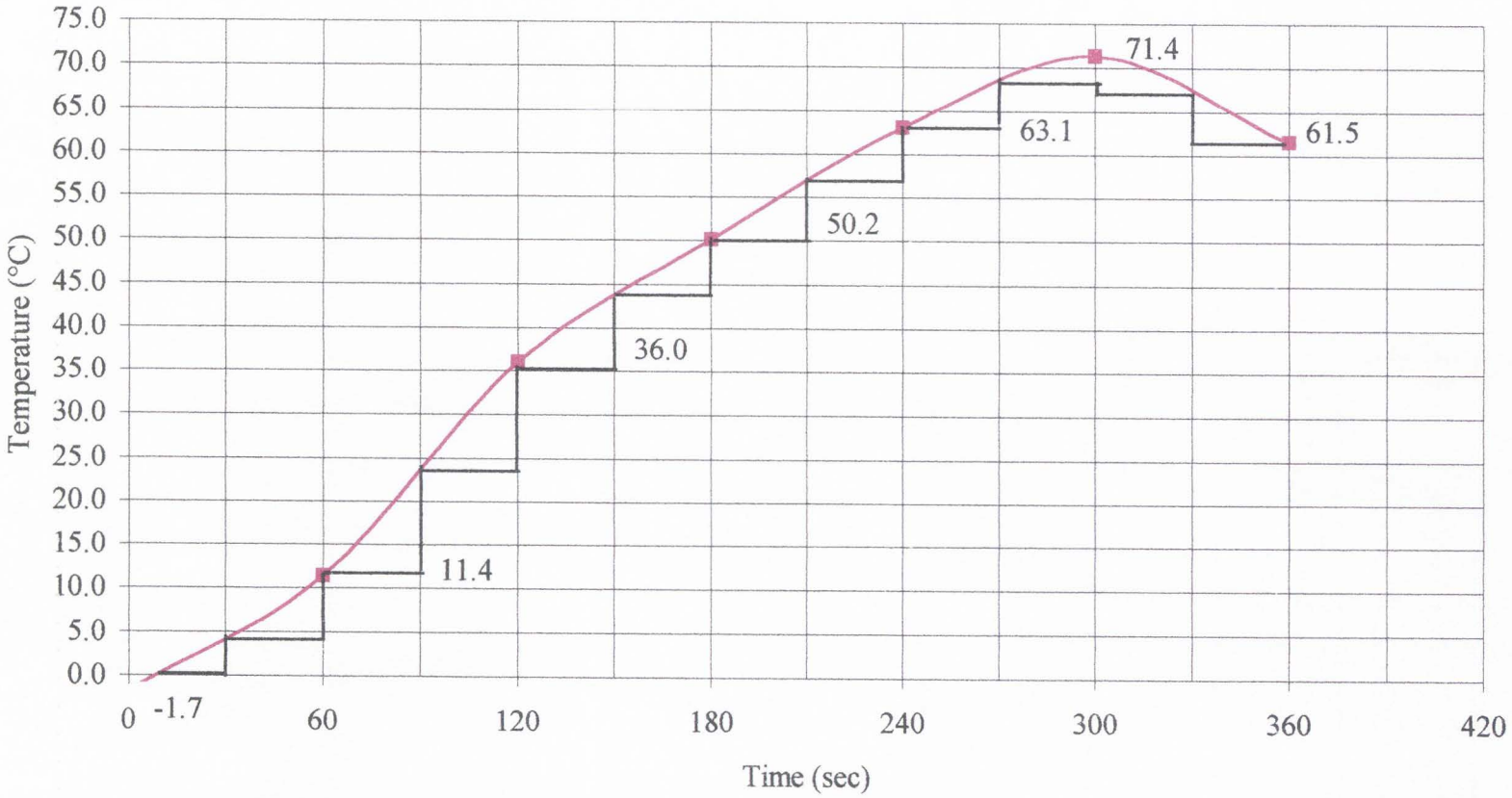


Fig. 8 -- Internal temperature at 30-sec intervals during cooking of lean hamburgers.
(Grill temperature - 165° C; pooled for all pHs; n=30). Hamburgers were removed from the grill when internal temperature reached 71°C.

Table 5 — Calculation of the cumulative F-value for 6-log destruction of *Escherichia coli* O157:H7 in extra lean hamburgers cooked to 71°C internal temperature by double-side frying (165°C grill; flipped when hamburger internal temperature ≥ 40°C)

Hamburger internal temperature (°C)	Holding time per temperature increment (sec)	Calculations (°C)	Calculations (°C)	$F_{71.1°C}^{4.78°C}$ - value per temperature increment[1] (sec)
T	D	(71.1 - T)	$10^{-(71.1-T)/4.78}$	$Dx10^{-(71.1-T)/4.78}$
6.0[2]	30	65.1	2.40300E-14	2.75083E-13
13.4	30	57.7	8.48927E-13	9.71810E-12
24.0	30	47.1	1.40102E-10	4.20305E-09
34.0	30	37.1	1.73178E-08	1.36152E-06
43.0	30	28.1	1.32233E-06	3.96698E-05
49.5	30	21.6	3.02811E-05	0.001212874
58.0	30	13.1	0.001817248	0.033676772
65.7	30	5.4	0.074181035	0.636035106
66.6	30	4.5	0.114439883	6.738848454
62.0	30	9.1	0.012480567	4.162753254
56.6	30	14.5	0.000925821	0.294274787
			Cumulative $F_{71.1°C}^{4.78°C}$:	**6.12** > 0.16[3]

1 Values calculated using Eq. 28 ($F = D_1 \times 10^{-(T^*-T)/z} + D_2 \times 10^{-(T^*-T)/z} + \ldots$).

2 Values are mean (n = 30) internal extra lean hamburger temperature at the beginning of each 30-sec interval during cooking to 71°C (from Fig. 7).

3 $F_{71.1°C}^{4.78°C}$ = 0.16 sec; i.e., 0.16 sec at 71.1°C (z = 4.78°C) is sufficient for 6-D destruction of viable *Escherichia coli* O157:H7 in extra lean (7% fat) ground beef (derived from data of Ahmed et al., 1995).

Table 6 — Calculation of the cumulative F-value for 6-log destruction of *Escherichia coli* O157:H7 in lean hamburgers cooked to 71°C internal temperature by double-side frying (165°C grill; flipped when hamburger internal temperature ≥ 40°C)

Hamburger internal temperature (°C)	Holding time per temperature increment (sec)	Calculations (°C)	Calculations (°C)	$F_{71.1°C}^{4.35°C}$ - value per temperature increment[1] (sec)
T	D	(71.1 - T)	$10^{-(71.1-T)/4.35}$	$D \times 10^{-(71.1-T)/4.35}$
4.0[2]	30	67.1	3.75589E-16	1.12677E-14
11.4	30	59.7	1.88739E-14	5.66218E-13
24.0	30	47.1	1.48735E-11	4.46206E-10
36.0	30	35.1	8.53168E-09	2.55950E-07
43.0	30	28.1	3.46920E-07	1.04076E-05
50.1	30	21	1.48735E-05	0.000446206
57.0	30	14.1	0.000573615	0.017208458
63.1	30	8	0.014485035	0.434551041
68.0	30	3.1	0.193801138	5.814034126
67.0	30	4.1	0.114148777	3.424463323
61.5	30	9.6	0.006210169	0.186305083
			Cumulative $F_{71.1°C}^{4.35°C}$:	**9.88** > 0.17[3]

1 Values calculated using Eq. 28 ($F = D_1 \times 10^{-(T^*-T)/z} + D_2 \times 10^{-(T^*-T)/z} + \ldots$).

2 Values are mean (n = 30) internal lean hamburger temperature at the beginning of each 30-sec interval during cooking to 71°C (from Fig. 8).

3 $F_{71.1°C}^{4.35°C} = 0.17$ sec; i.e., 0.17 sec at 71.1°C (z = 4.35°C) is sufficient for 6-D destruction of viable *Escherichia coli* O157:H7 in lean (20% fat) ground beef (derived from data of Ahmed et al., 1995).

Table 7 — Calculation of the cumulative F-value for 6-log destruction of *Salmonella* in extra lean hamburgers cooked to 71°C internal temperature by double-side frying (165°C grill; flipped when hamburger internal temperature ≥ 40°C)

Hamburger internal temperature (°C)	Holding time per temperature increment (sec)	Calculations (°C)	Calculations (°C)	$F_{71.1°C}^{5.56°C}$ - value per temperature increment[1] (sec)
T	D	(71.1 - T)	$10^{-(71.1-T)/5.56}$	$Dx10^{-(71.1-T)/5.56}$
6.0[2]	30	65.1	1.95599E-12	5.86797E-11
13.4	30	57.7	4.19085E-11	1.25726E-09
24.0	30	47.1	3.37891E-09	1.01367E-07
34.0	30	37.1	2.12490E-07	6.37469E-06
43.0	30	28.1	8.83168E-06	0.000264950
49.5	30	21.6	0.000130349	0.003910472
58.0	30	13.1	0.004404381	0.132131434
65.7	30	5.4	0.106850603	3.205518097
66.6	30	4.5	0.155112744	4.653382334
62.0	30	9.1	0.023083909	0.692517268
56.6	30	14.5	0.002466530	0.073995888
			Cumulative $F_{71.1°C}^{5.56°C}$:	**8.76** > 0.12[3]

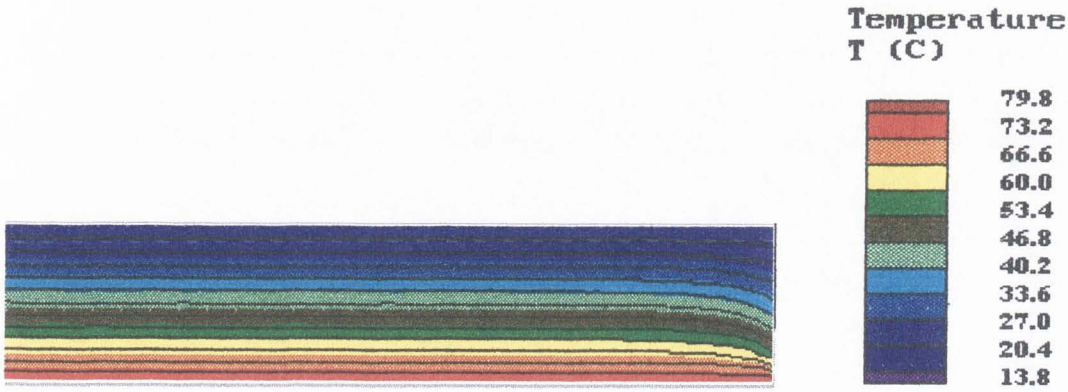
1 Values calculated using Eq. 28 ($F = D_1 \times 10^{-(T^*-T)/z} + D_2 \times 10^{-(T^*-T)/z} + \ldots$).

2 Values are mean (n = 30) internal extra lean hamburger temperature at the beginning of each 30-sec interval during cooking to 71°C (from Fig. 7).

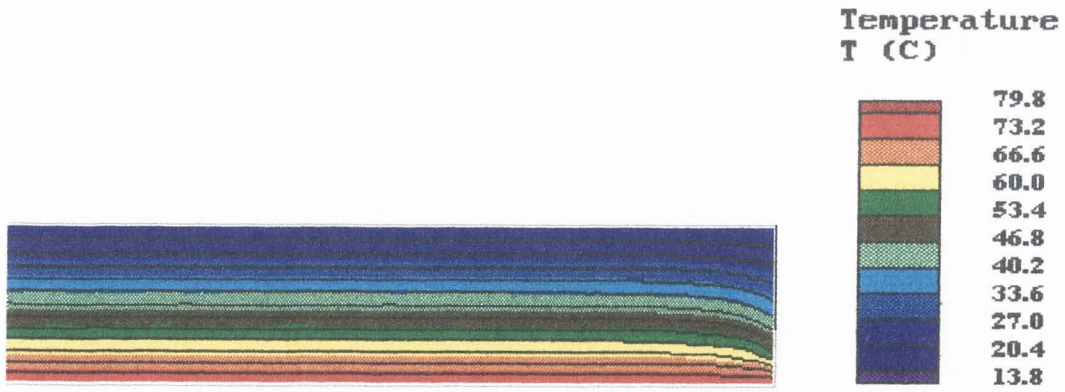
3 $F_{71.1°C}^{5.56°C} = 0.12$ sec; i.e., 0.12 sec at 71.1°C (z = 5.56°C) is sufficient for 6-D destruction of viable *Salmonella* in ground beef (derived from data of Goodfellow and Brown, 1978).

Finite-element model of temperature distribution

The finite-element model was built to analyze steady-state heat transfer in extra lean and lean hamburgers, using the QuickField ™ program (Tera Analysis Co., Tarzana, CA). Postprocessing results were presented as an isotherm color (Fig. 9 and Fig. 10). Eleven differently colored temperature layers of hamburger with a 6.6°C interval were observed at the moment of hamburger flipping, from 79.8°C at the bottom crust to 13.8°C on the top surface. The top surface was only slightly affected by heat, with a little skewness of temperature isotherms on the circumferential surface. The predicted temperatures of 42.5 and 44.2°C in the geometrical center of extra lean and lean hamburger were almost identical to the experimental overall mean temperatures of 42.4 and 44.4°C, respectively (Table 3). At the end point of frying, 11 differently colored temperature layers of hamburger were observed, starting from 79.8 and 80.8°C at the bottom crust and ending at 45.8 and 44.8°C on the top surface of extra lean and lean hamburgers in 3.4 or 3.6°C intervals, respectively. A slightly lower temperature was observed on the bottom surface of extra lean hamburger, compared with lean hamburger. Perhaps without much melted beef fat, the extra lean hamburgers develop a thicker surface "skin" and have less surface heat transfer from the grill. However, extra lean hamburger had higher top surface temperature than lean hamburger as a result of higher thermal conductivity of water, compared to beef fat. Predicted temperatures of 63.2 and 63.8°C in the geometrical center of hamburger were 12% lower than experimental overall mean temperatures of 72.4 and 72.0°C for extra lean and lean

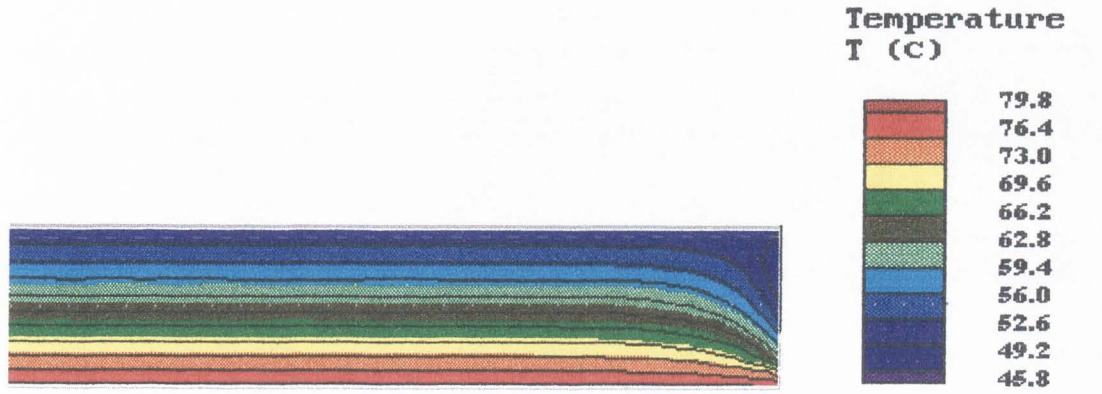


(a)

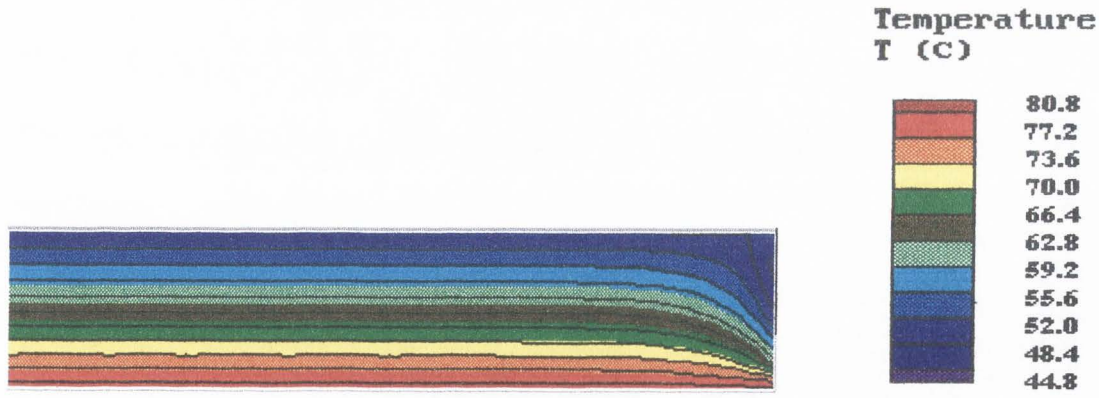


(b)

Fig. 9 -- Temperature distribution in extra lean (a) and lean hamburger (b) at the moment of flipping (isotherm lines shown at 5°C interval).



(a)



(b)

Fig. 10 -- Temperature distribution in extra lean (a) and lean hamburger (b) at the end of cooking (isotherm lines shown at 3°C interval).

hamburgers, respectively (Table 3). Similar lower predicted than actual temperatures were reported at the center for oven roasting of meat balls by Holtz and Skjoldebrad (1986), who also used the FEM. Dagerskog (1979) and Holtz and Skjoldebrand (1986) reported maximum discrepancies between observed and predicted temperatures of 15 and 17°C during double-sided pan frying and oven roasting of meat patties, respectively.

The coldest spot was observed on the circumferential surface of hamburgers. Predicted values of the model for the coldest spot were 39.9 and 40.1°C at the moment of flipping and 45.8 and 44.8°C at the end point of cooking for extra lean and lean hamburgers, respectively. The circumferential coldest spot has a very small area and may not occur in reality. The model assumes no mass transfer and deformation during cooking, but of course mass transfer and shrinkage or expansion does occur (Table 8). It is very difficult to measure an accurate temperature at this edge with thermocouple. Also, migration of hot meat juice and melted fat during cooking in absence of crust may increase predicted temperatures significantly. However, the photograph of extra lean hamburgers of pH > 6.5 cooked to 71°C internal temperature shows a red ring of undenatured myoglobin (Fig. 11), indicating the possibility of existence of such a cold spot on the mid-plane, and not at the geometrical center. The red circumferential ring of extra lean hamburgers of pH > 6.5 is possibly due to less deformation of hamburgers during cooking and, as a result, highest cooking yield. High cooking yield of dark-cutting hamburgers was related to high water binding and low level of fat migration at elevated meat pH. A red circumferential ring was not observed in hamburgers shrunk less in diameter and had less expansion in height. There was no

Table 8 — Physical characteristics of extra lean and lean hamburgers of pH = 5.80-6.73 cooked to 71°C internal temperature

Fat level	Raw meat pH	Initial weight (g)	Initial density (kg/m^3)	Cooked weight (g)	Cooked density (kg/m^3)	Cooking time (sec)	Yield (%)	Diameter shrinkage (%)	Thickness expansion (%)	Penetration load (g)
Extra lean[1]	5.80	126.0±4.5	1114±41	112.0±7.0	1126±60	244±37	88.7±3.9	6.6±1.8	1±7	569±59
Extra lean	6.29	117.6±4.7	1039±42	110.3±4.5	1054±37	216±31	93.8±2.1	4.7±1.2	2±4	725±76
Extra lean	6.73	118.9±4.4	1051±39	113.6±4.5	1043±51	228±25	95.4±1.2	5.5±1.3	8±6	1022±178
Overall mean		120.8±5.8[a]	1068±51[a]	111.9±5.5[a]	1074±61[a]	230±32	92.7±3.9[a]	5.6±1.6[b]	4±7[b]	772±222[a]
Lean	5.80	121.9±4.0	1077±35	103.4±4.5	1026±79	248±14	84.8±3.1	8.5±1.3	7±7	479±61
Lean	6.29	115.2±4.4	1018±39	105.4±4.2	988±44	227±20	91.5±1.2	7.2±1.0	10±5	648±146
Lean	6.73	116.9±4.7	1033±41	107.3±4.9	970±76	229±24	91.7±1.9	7.6±2.7	15±7	895±113
Overall mean		118.0±5.1[b]	1043±45[b]	105.4±4.7[b]	995±70[b]	234±21	89.3±3.9[b]	7.8±1.9[a]	11±7[a]	673±206[b]
Fisher's LSD		2.0	17.7	2.5	35.8	15.8	0.9	0.7	2	71

[1] Values are means (n = 10 and n = 30 for overall mean) ± standard deviation.

[a-b] Overall means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Fig. 11 -- Side view of extra lean hamburgers from dark-cutting beef pH 6.59-6.92 cooked to 71°C internal temperature.

cooked lean or extra lean hamburgers of pH = 5.80-6.29. The discovery of the coldest spot on the circumferential surface of extra lean hamburgers from dark-cutting beef agrees with the results of the mathematical model developed by Ikediala et al. (1996) for transient heat transfer in meat patties during single-sided pan frying. The presence of the cold spot that does not reach the required 71.1°C end temperatures during double-side frying raises a question about the microbial safety of extra lean hamburgers from dark-cutting beef, using normal frying operations.

Physical properties of hamburgers

Initial weight and density of hamburgers of pH 5.80 were higher than at pH 6.29 and 6.73 (Table 4). There was no difference between cooking time and cooked weight of hamburgers between pH groups. The highest cooked yield and lowest cooked density were observed for hamburgers with elevated pH. The densities calculated for raw hamburgers are in agreement with reported density of uncooked bovine skeletal muscle of 1060-1070 kg/m^3 (Klein et al., 1969; Jarvis, 1971). Compared to pH 5.80, hamburgers of pH 6.29 and 6.73 had less shrinkage in diameter and higher thickness expansion after cooking. The results of a penetration test showed that with increasing pH, bind strength of hamburgers increased significantly (Table 66, Appedix B).

Extra lean hamburgers had significantly higher initial weight and density, cooked weight and density, cooking yield, and bind strength than lean hamburgers (Table 8; Table 58, Appedix B). Compared to 20% fat hamburgers, extra lean

interaction between physical parameters of hamburgers at different pH groups and fat levels. These results agree well with Troutt et al. (1992b), who observed that extra lean beef patties (5% fat) of normal pH had higher initial weight, low cooking losses, higher firmness, and different deformation pattern compared with lean beef patties (30% fat).

CONCLUSIONS

There was no pH effect on cooking-temperature profile and cooking time for extra lean (3.3% fat) and lean hamburgers (20% fat) with different pH. Hamburgers had the same homogeneous temperature of 60°C at 210-220 sec after cooking started, as shown by the intersection point of the three temperature-time curves. The highest cooked yield and lowest cooked density were observed for hamburgers with elevated pH. Hamburgers were adequately cooked for greater than 6-log destruction of viable *E. coli* O157:H7 and *Salmonella*, except for the cold spot on the circumferential surface of hamburgers, which did not reach the required 71.1°C. A double-sided pan fryer would probably give better heat penetration for high pH extra lean hamburgers.

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CHAPTER IV

EFFECT OF MEAT PH AND FAT CONTENT ON PINK COLOR RETENTION IN COOKED HAMBURGERS

ABSTRACT

The effect of pH (5.80, 6.29, 6.73) on myoglobin denaturation in extra lean (3.3% fat) and lean (20.0% fat) hamburgers was studied. Compared to normal meat (pH = 5.8), raw extra lean ground beef of pH = 6.73 had significantly lower oxidation-reduction potential value of -267.6±88.8 mV, lower concentration of metmyoglobin after 48 hr of refrigerated storage, and more distinct cherry-red color. There was no significant pH effect on external color of cooked hamburgers. However, the internal color of cooked hamburgers from meat of pH = 6.73 was distinctly darker (L = 37.7), redder (a = 6.0), and lighter yellow (b = 8.8), with low saturation index (11.0) and hue angle (55.3) values. Hamburgers of pH = 6.29 were intermediate in color characteristics. The internal color of cooked lean hamburgers was lighter (L = 43.9), less red (a = 4.3), and more yellow (b = 11.0), with higher saturation index (11.9) and hue angle (68.6), compared with extra lean hamburgers (L = 37.1, a = 5.6, b = 9.5, saturation index = 11.3, and hue angle = 58.7, respectively). Hamburgers of pH = 6.29 and 6.73 had greater levels of undenaturated myoglobin concentrations (3.1 and 3.8 mg/g) and lower percent myoglobin denaturation (59.9 and 47.9%) than hamburgers of pH = 5.80 (2.0 mg/g and 67.9%, respectively). Percentage of myoglobin denaturation during cooking was not affected by total pigment or fat content of hamburgers. However, cooked

extra lean hamburgers with high total pigment looked more red than lean normal hamburgers. Neither pH nor fat content affected metmyoglobin as percentage of total pigments in cooked hamburgers. The pH $\geq$ 6.5 and oxidation-reducing potential $\leq$ -200 mV are characteristics of dark-cutting beef.

INTRODUCTION

It has been estimated that the dark-cutting meat condition cost the beef industry approximately $132.5 million in 1991, or approximately $5 for every steer and heifer slaughtered (Smith et al., 1992). The incidence of dark-cutting carcasses with high pH is estimated at 3.2-5.2 % in Ireland (Tarrant and Sherington, 1980), 8% in Canada, and 0.33-4.7% by several investigators in the USA (Munns and Burrell, 1966). Beef is dark-cutting when the final pH value of the meat is above 6.0 (Tarrant, 1987). This condition is caused by an absence of glycogen in the muscles at death. High muscle glycogen breakdown antemortem and low lactate concentration postmortem result from trauma, isolation, or psychological stress (Apple et al., 1995). At high postmortem muscle pH, mitochondria are active at high pH and consume surface oxygen, keeping myoglobin deoxygenated, thus preventing development of the normal red color of meat (Cornforth and Egbert, 1985; Cheah and Cheah, 1970). The major muscles of the loin and round are the most severely affected and have the highest pH of 13 major muscles (Tarrant and Sherington, 1980). Utilization of dark-cutting beef is a problem to meat producers and processors, for two reasons. First, dark-colored retail beef cuts are usually rejected by

consumers. Secondly, dark meat of high pH often has a raw, undercooked appearance after normal cooking.

However, one benefit of high pH meat may be its greater resistance to oxidation and surface browning during retail display. Chow (1991) stated a relationship between the stability against denaturation and autoxidation of fish myoglobin: the higher the stability of Mb against denaturation, the lower the autoxidation rate constant. Patties made from high pH meat would not also have the problem of "premature browning," recently described by researchers at Kansas State University. Warren et al. (1996) reported that oxidation of myoglobin to metmyoglobin produced premature brown color of cooked beef patties at cooking temperatures low as 55°C.

In a study by Schmidt and Trout (1984), meat slurries of beef, pork, and turkey were pH-adjusted to 5.5, 6.0, and 6.5 and cooked to 62.8, 68.3, or 73.9°C. Even when cooked to the same internal temperature, the high pH beef, pork, and turkey were redder than the low pH meats. The authors suggested the reason for this phenomenon was that high pH meats protect myoglobin from being denatured. Trout (1989) confirmed that the pH effect was the greatest at lower temperatures (55 and 62°C) where the percentage of denatured myoglobin was 3 to 14 times greater at pH 5.5 than at pH of 7.0. Denaturation of meat pigments could be increased by lowering the pH and increasing cooking temperature. Metmyoglobin appears to be more resistant to heat denaturation than either myoglobin or oxymyoglobin, especially at high pH (Janky and Froning, 1973). Hard-to-cook hamburgers, characterized by persistent internal red color during cooking, were

associated with high pH raw meat, such as bull meat (Mendenhall, 1989). Pink or red colors of meat pigments only occur under reducing conditions. In fresh meat, heme iron must be reduced (ferrous) in order for oxygen binding or bloom to occur. In cooked meats, heme is exposed due to globin denaturation, and the heme iron is more rapidly oxidized than in fresh meats upon exposure to air. Thus, relatively strong reducing conditions are needed for stable pink complexes to occur in cooked meat. The oxidation-reduction potential at which pink color appeared upon titration with a strong reductant ranged from -321 to -511 mV (Cornforth et al., 1986).

Meat color is one of the most important factors affecting retail meat purchase decisions (Kropf, 1980). Consumers are suspicious of any muscle or meat color abnormalities (Romans and Ziegler, 1977). Hamburgers from dark-cutting beef stay red or pink inside, even if an internal end-point temperature of 71.1°C was reached. For both consumers and food service, cooking guidelines suggest that beef hamburgers be cooked to 71.1°C or until no pink color remains in the center, and juices are clear (USDA, 1993; USDA-FSIS, 1993). The purpose of this rule is to eliminate *E. coli* and *Salmonella* food poisoning caused by the consumption of undercooked beef. Most consumers expect cooked beef hamburgers to be grey inside. They often interpret red or pink color in beef products as an indication of undercooking.

Most of the research related with high pH of meat and denaturation of myoglobin was done in vitro by adjusting of ground meat pH by NaOH or lactic acid (Schmidt and

Trout, 1984; Trout, 1989, 1990), or at pH of beef patties that did not exceed 6.2 (Mendenhall, 1989; Van Laack et al., 1996).

The objective of this study was to determine the effect of total meat pigment, oxidation-reduction potential, and total reducing ability of raw ground beef on percent myoglobin denaturation by frying of 3% and 20% fat hamburgers of pH 5.6-6.9 to 71°C internal temperature.

MATERIALS AND METHODS

Preparation of beef hamburgers

Hamburgers were prepared as previously described in Chapter III.

Moisture, fat, and pH measurements

Moisture and fat were measured as previously described in Chapter III.

Meat pigment measurements

Pigment identification was done by spectrophotometric procedures as described by Cornforth (1991). Myoglobin, metmyoglobin, and total pigment content were calculated based on absorbance of clarified extract at 525, 572, and 700 nanometers for raw and cooked meat (Trout, 1989) using a Shimadzu model UV-2100 UV-VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan). Heme pigments were extracted using 0.04 M phosphate buffer at pH 6.8 (Warriss, 1979). Myoglobin and metmyoglobin concentration was calculated using the following formula (Krzywicki, 1979):

$$\text{Myoglobin (mg / mL)} = (A_{525} - A_{700}) \text{ x } 2.303 \text{ x dilution factor} \quad (35)$$

$$\text{Metmyoglobin (\%)} = \left(\frac{1.395 - (A_{572} - A_{700})}{(A_{525} - A_{700})} \right) \text{ x } 100 \quad (36)$$

where A_λ = Absorbance at wavelength λ, in nanometers (nm). The percentage of myoglobin denatured (PMD) was calculated using the following formula:

$$\text{PMD (\%)} = \left(1 - \frac{\text{myoglobin conc after heating}}{\text{myoglobin conc before heating}} \right) \text{ x } 100 \quad (37)$$

Total pigment concentration was calculated using the following formula (Hornsey, 1956):

$$\text{Total pigment (ppm)} = 135.8 \text{ x } A_{640} \text{ x total vol. (mL) / sample (g)} \quad (38)$$

where A_{640} = Absorbance at 640 nm. The factor 0.026 (Franke and Solberg, 1971) was used to convert ppm hematin to total pigment (mg/g). This factor was calculated by dividing the molecular weight of myoglobin ($\approx$ 17, 000) by the molecular weight of hematin (656) x 10^3.

Hunter color measurements

Surface color of raw patties, and crust and internal color of cooked hamburgers were quantitated with the Hunter Lab Digital Color Difference Meter (D25D2A), standardized using white (L = 94.9, a = -0.9, and b = 2.0) and black (Y = 0.00, X = 0.00, and Z = 0.00) standard plates. Surface color of raw and cooked hamburgers was measured from both sides at room temperature immediately after production or cooking. Internal surface color readings of cooked hamburgers was taken immediately after cutting them longitudinally. Two patties were used per experimental unit. The hue-angle = $(b/a)^{\tan-1}$

may better define redness of meat than the Hunter color a-value. The larger hue-angle of meat, the less red color is present (Van Laack et al., 1996). Hue-angle corresponds to color in a 360° Munsell color wheel. Saturation index corresponds to color intensity.

Oxidation-reduction potential and total reducing ability measurements

A modified method of Galesloot and Kooy (1960) for measuring oxidation-reduction potential (ORP) in cheese and milk was used as a prototype for ORP measurement in raw meat. ORP of raw beef was measured with an platinum redox electrode (Orion, Model 96-78-00) inserted in the center of a raw extra lean ground beef chub (0.5 kg; 3-5°C). The electrode was sealed by the sticky meat and wrapped with food grade polyvinyl chloride film in order to prevent meat from drying during measurements. Chubs were placed inside an isolated foam chest with several "blue ice" cooling packs to maintain temperature < 5°C. A temperature-reading sensor was also inserted to monitor temperature of meat. The redox electrode and temperature sensor were calibrated according to the manufacturer's instructions. ORP readings (mV) were taken using an Orion pH meter model 420A (Orion Inc., Cambridge, MA) for 24-48 hr at 20-min intervals. The pH meter was connected to an IBM-compatible computer by a standard RS 232 cable. ORP, temperature, and time data were transferred and collected on a computer by the communication program Terminal for Windows 3.1 (Microsoft Corp., Redmond, WA).

Total reducing ability was measured as describe by Lee et al. (1981; Appendix C).

Electric grill and frying operation

Hamburgers were fried using a Hotpoint electric grill and frying operation as previously described in Chapter III.

Experimental design and statistical analysis

Data were analyzed as a completely randomized design. Treatments were arranged as a 3 x 2 factorial in a split-plot design, where three pH groups — normal beef (pH ≤ 6.0), mild dark-cutting beef (pH = 6.01-6.49), and extreme dark-cutting beef (pH = 6.50-6.92) — were as the whole plot factor with two levels of fat — extra lean (≤ 5% fat) and lean (about 20% fat) — as subplot factors. Five replications were performed per treatment, and two hamburgers were used for each experimental unit.

Experimental data were analyzed using the Statistical Analysis System program (SAS, 1988). ANOVA, GLM, Pearson correlation, and multiple comparison Fisher's LSD values ($p<0.05$) were used for statistical analysis of proximate analysis, meat pigments, pH, ORP, total reducing ability, and Hunter color parameters of hamburgers (Tables 67-97, Appendix D).

RESULTS AND DISCUSSION

Chemical characteristics of raw meat

Extra lean ground beef from dark-cutting inside rounds was characterized (Table 9). Trimmed ground beef from inside rounds had 73.4±0.9% moisture, 3.3±0.9% fat and 21.4±1.2% protein. There were no significant differences for meat

pigment concentrations between pH groups (Tables 68-70, Appendix D). Mean myoglobin concentration (deoxymyoglobin + oxymyoglobin + metmyoglobin) of extra lean hamburgers was 7.7±1.2 mg/g. Metmyoglobin was 20.7±13.8 mg/g of total myoglobin concentration. Total acetone-extractable meat pigment concentration (mainly myoglobin and hemoglobin) was 7.6±0.4 mg/g. Thus, there was little if any residual blood hemoglobin in the meat. Meat with elevated pH had lower reducing ability than meat with normal pH (Table 9; Table 72, Appendix D). The correlation between raw meat pH and reducing ability was high -0.82 ($p = 0.0001$; Table 10). This could be explained as a depletion of reducing agents (glycogen, glucose) in dark-cutting beef. Oxidation-reduction potential (ORP) values of raw extra lean meat were reported at 24 hr (Fig. 12; Table 11). Meat at extreme high pH had significantly lower mean ORP value of -267.6±8 8.8 mV than other groups (Table 73, Appendix D). The correlation between raw meat pH and ORP was also high ($r = -0.81$; $p = 0.0005$; Table 10). A low meat ORP may contribute to the known heat stability of Mb at high pH. Reducing ability and ORP significantly correlated between each other ($r = 0.72$; $p = 0.0005$). Low ORP and reducing ability of raw meat characterize dark-cutting beef. The addition of beef fat trim to extra lean meat to adjust fat level to 20% could be considered as a simple dilution factor (17-15%) that changed only the composition proportions of the meat.

A separate experiment was done to check the rate of metmyoglobin formation in extra lean ground beef after 48 hr storage at 3°C (Table 12; Tables 74-75,

Table 9 — Compositional characteristics of extra lean and lean hamburgers

Fat level	pH group	Raw meat pH	Moisture (%)	Fat (%)	Protein[1] (%)	Myoglobin (mg/g)	Met-myoglobin (%)	Total pigment (mg/g)	ORP[2] at 24 hr (mV)	Total reducing ability
Extra lean	1[3]	5.80±0.12^{a}	73.0±1.1	3.3±0.9	21.8±1.2	7.0±0.4	29.1±19.4	7.3±0.6	-111.0±22.0^{a}	0.44±0.04^{a}
Extra lean	2	6.29±0.14^{b}	73.4±0.7	3.3±0.9	21.4±1.2	8.2±1.0	17.2±6.6	8.0±1.0	-159.8±97.3^{a}	0.38±0.05^{b}
Extra lean	3	6.73±0.13^{c}	73.8±0.8	3.3±0.9	21.0±1.1	7.8±1.7	16.2±9.0	7.5±1.5	-267.6±88.8^{b}	0.33±0.03^{b}
Overall mean		6.27±0.41	73.4±0.9^{a}	3.3±0.9^{b}	21.4±1.2^{a}	7.7±1.2^{a}	20.7±13.8	7.6±0.4	-179.5±95.8	0.38±0.06
Lean	1	5.80±0.12^{a}	61.2±1.2	19.8±1.3	17.0±2.1	5.8±0.5	36.0±23.9	---	---	---
Lean	2	6.29±0.14^{b}	60.6±1.3	20.3±1.0	17.1±1.5	6.9±1.0	18.6±9.5	---	---	---
Lean	3	6.73±0.13^{c}	61.2±1.0	20.0±1.2	16.9±1.7	6.7±1.6	19.4±11.0	---	---	---
Overall mean		6.27±0.41	61.0±1.2^{b}	20.0±1.2^{a}	17.0±1.7^{b}	6.5±1.2^{b}	24.7±17.6	---	---	---
Fisher's LSD		0.2	0.8	0.3	0.9	0.2	4.7	1.6	106.3	0.06

1 Percent protein was calculated by difference using typical beef composition for carbohydrates (1.2%) and inorganic salts (0.7%; Lawrie, 1968).

2 Oxidation-reduction potential values after 24 hr in ground beef chubs at 3°C.

3 Group 1 - pH ≤ 6.00; group 2 - pH = 6.01-6.49; group 3 - pH ≥ 6.50.

abc Means (n = 10 and n = 30; ± standard deviation) with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 10 — Pearson correlation coefficients (r) among Hunter color values, pigment concentration, and chemical characteristics of extra lean hamburger main characteristics

	PMD[1]	Undena-turated Mb[2]	Cooked meat inside Hunter "a"	Cooked meat inside Hue angle	Raw meat pH	Raw meat total pigment	Raw meat Mb[2]	Raw meat MetMb[3]	Raw meat total reducing ability	Raw meat ORP[4]
PMD[1]	1.00									
Undenatured Mb	-0.91[a]	1.00								
Cooked meat inside Hunter "a"	-0.87[a]	0.81[a]	1.00							
Cooked meat inside Hue angle	0.87[a]	-0.83[a]	-0.95[a]	1.00						
Raw meat pH	-0.72[a]	0.64[a]	0.77[a]	-0.83[a]	1.00					
Raw meat total pigment	n.s.[5]	0.36[b]	n.s.	n.s.	n.s.	1.00				
Raw meat Mb[2]	n.s.	0.59[a]	0.27[b]	-0.27[b]	n.s.	0.87[a]	1.00			
Raw meat MetMb[3]	0.44[a]	-0.26[b]	-0.32[b]	0.31[a]	-0.43[a]	0.35[a]	n.s.	1.00		
Raw meat total reducing ability	0.52[a]	-0.44[a]	-0.49[a]	0.61[a]	-0.82[a]	n.s.	n.s.	0.27[b]	1.00	
Raw meat ORP[4]	0.61[a]	-0.41[a]	-0.66[a]	0.75[a]	-0.81[a]	0.32[b]	n.s.	0.45[a]	0.72[a]	1.00

[1-5] PMD - percentage myoglobin denaturation; Mb - myoglobin; MetMb - metmyoglobin; ORP - oxidation-reduction potential; n.s. - not significant $p > 0.05$ ($n = 30$), respectively.

[ab] Significance level - $p < 0.0005$ and $p < 0.05$, respectively.

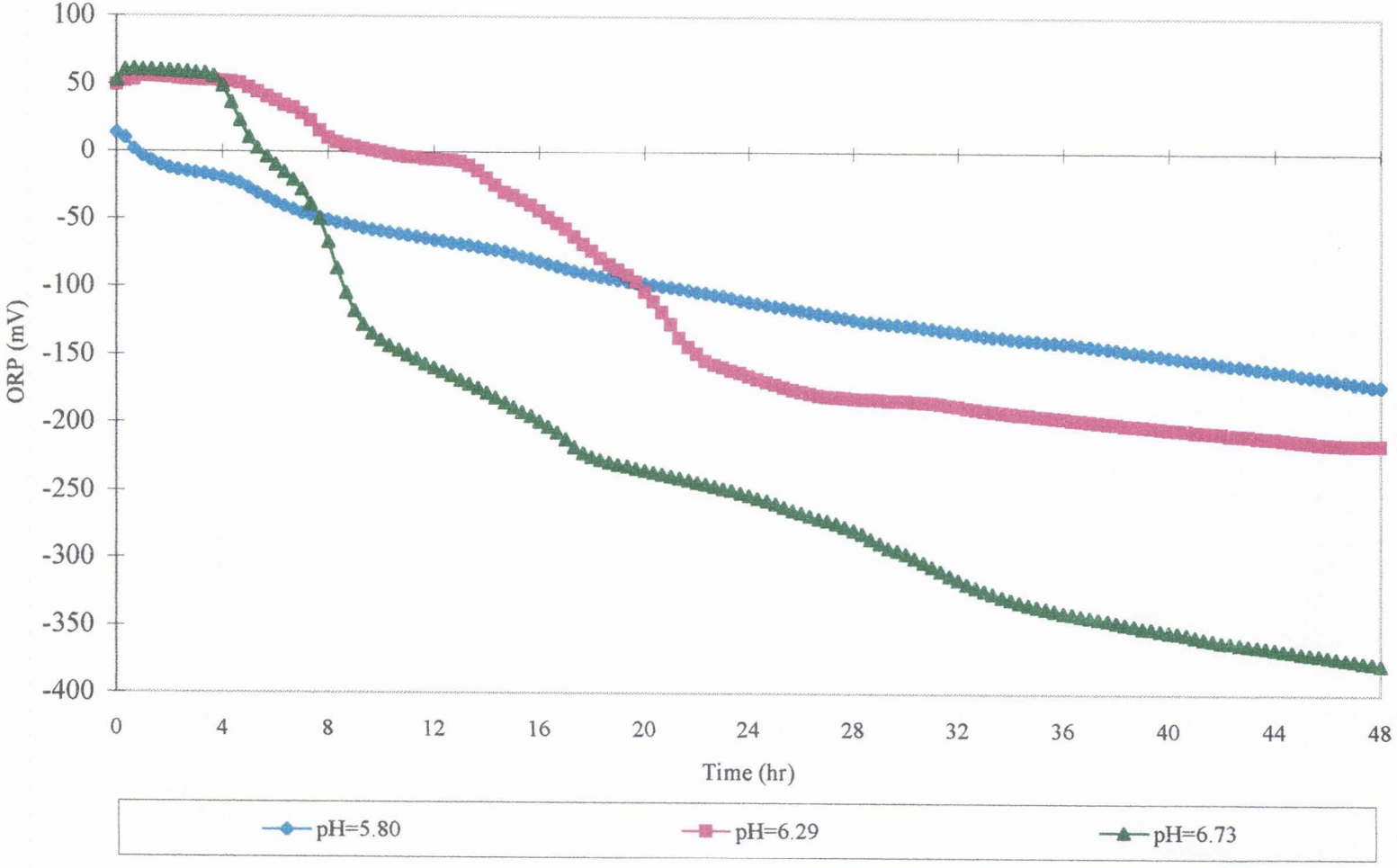

Fig. 12—Oxidation-reduction potential of extra lean ground beef during storage at 3°C (n=3).

Table 11 — Mean oxidation-reduction potential values with time at 3°C in extra lean ground beef pooled for all pH groups

Time (hr)	Oxidation-reduction potential (mV)[1]
0	44.0 ± 22.3^{a}
5	0.2 ± 64.2^{ab}
10	-74.0 ± 72.9^{b}
24	-179.5 ± 98.4^{c}
48	-250.5 ± 121.3^{c}
Fisher's LSD	76.8

[1] Values are means (n = 9) ± standard deviation.

[abc] Means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Appendix D). Concentration of metmyoglobin (MetMb) almost doubled after 48 hr of storage for meat of pH = 5.80, and did not changed for meat of pH = 6.73. These results are in agreement with Ledward et al. (1977), who found that oxidation of oxymyoglobin innormal pH minced beef during storage at 1°C depended from pH in the 5.35-6.15 range. The rate constants for different muscles, although being pH dependent, were similar for all major beef muscles. When the enzymatic MetMb reducing system is destroyed by mincing, the formation of MetMb is at least 10-fold more rapid than in intact meat (Ledward et al., 1977; Ledward, 1972). The rate of autoxidation decreased with

increasing pH, while the rate of reduction was believed to increase with increasing pH (Ledward, 1985). Bembers and Satterlee (1975) also found that Mb extract from pale soft and exudative porcine muscle (acid pH) autoxidized at a rate approximately double that of an extract from normal 1977; Ledward, 1972). The rate of autoxidation decreased with increasing pH, while the muscle. Freezing and thawing of meat caused autoxidation of bluefin tuna myoglobin (MetMb, 15-40%) depending upon pH (Chow et al., 1987). The results of this experiment also indicate that ground beef at higher pH is more resistant to oxidation of myoglobin to metmyoglobin (Table 12).

Table 12 — Effect of pH and storage time at 3°C on myoglobin and metmyoglobin concentration in extra lean ground beef

Raw meat pH	Time of storage (hr)	Myoglobin[1] (mg/g)	Metmyoglobin[1] (%)
5.80	0	7.20 ± 0.35	23.08 ± 10.42[b]
5.80	48	6.79 ± 0.36	40.16 ± 11.57[a]
Fisher's LSD		0.64	5.85
6.73	0	9.16 ± 0.89	22.20 ± 6.65
6.73	48	9.01 ± 0.94	21.60 ± 6.70
Fisher's LSD		0.64	5.85

[1] Values are means (n = 6) ± standard deviation.

[ab] Means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Raw meat color

Results of Hunter color measurements of raw meat are presented in Table 13 and Table 14. After freezing, thawing, and grinding, dark-cutting beef bloomed very well (Fig. 13), producing distinct and attractive cherry-red color with a significantly higher a-value than hamburgers from normal meat (a = 18.7 versus 14.6, respectively; Tables 76-81, Appendix D). If mitochondrial respiration is inhibited, myoglobin of dark-cutting beef muscle becomes oxygenated, and meat turns red (Egbert and Cornforth, 1986). In this study, the a/b ratio and saturation index were higher, and hue angle was lower for dark-cutting beef than for normal beef (Table 9). Lean hamburger was lighter (L = 38.0) and more yellowish (b = 12.7), with higher saturation index (21.5) and hue angle (36.5) values, than extra lean hamburgers (L = 28.5, b = 9.2, saturation index = 19.2, and hue angle = 9.3, respectively). There were no interactions between fat level and pH of Hunter color values.

External and internal surface color of cooked hamburgers

All hamburgers were cooked to 71°C internal temperature and cooking time (230±30 sec) was the same among fat levels or pH groups. Hunter color characteristics of the outside surface of cooked extra lean and lean hamburgers are presented in Table 15. External surface color of cooked lean hamburgers was lighter color (L = 37.2) than extra lean hamburgers (L = 34.0). There were no significant differences in external surface color between pH groups (Tables 82-87, Appendix D).

Table 13 — Hunter color parameters of raw hamburgers at different pH

Raw meat pH	L	a	b	a/b	Saturation index[2]	Hue angle[2]
5.80[1]	33.6 ± 5.1	14.6 ± 2.5^{b}	10.7 ± 2.0	1.4 ± 0.3^{b}	18.2 ± 2.5^{b}	36.4 ± 6.0^{a}
6.29	32.6 ± 5.1	17.9 ± 2.4^{a}	10.9 ± 2.0	1.7 ± 0.3^{a}	21.0 ± 2.7^{ab}	31.3 ± 4.2^{b}
6.73	33.5 ± 5.4	18.7 ± 2.1^{a}	11.3 ± 2.2	1.7 ± 0.3^{a}	21.9 ± 2.5^{a}	30.9 ± 4.5^{b}
Fisher's LSD	2.5	3.1	1.5	0.2	3.2	3.8

1 Values are means (n = 20) ± standard deviation.

2 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{\tan-1}$.

a-d Means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 14 — Hunter color parameters of raw extra lean and lean hamburgers by pH group

Fat level	Raw meat pH	L	a	b	a/b	Saturation index[2]	Hue angle[2]
Extra lean[1]	5.80	29.2 ± 1.4	14.5 ± 2.7	9.2 ± 1.2	1.6 ± 0.2	17.2 ± 2.8	32.6 ± 3.0
Extra lean	6.29	28.0 ± 1.3	17.5 ± 3.0	9.3 ± 1.2	1.9 ± 0.2	19.8 ± 3.0	28.3 ± 3.1
Extra lean	6.73	28.3 ± 0.7	18.2 ± 2.3	9.1 ± 0.8	2.0 ± 0.1	20.5 ± 2.4	26.8 ± 1.6
Overall mean		28.5 ± 1.2[b]	16.7 ± 3.1	9.2 ± 1.0[b]	1.8 ± 0.2[a]	19.2 ± 3.0[b]	29.3 ± 3.6[b]
Lean	5.80	38.0 ± 3.3	14.7 ± 2.4	12.3 ± 1.4	1.2 ± 0.2	19.2 ± 2.0	40.1 ± 5.9
Lean	6.29	37.2 ± 2.6	18.3 ± 1.5	12.5 ± 1.2	1.5 ± 0.1	22.1 ± 1.6	34.4 ± 2.6
Lean	6.73	38.7 ± 1.6	19.1 ± 1.8	13.3 ± 0.7	1.4 ± 0.1	23.3 ± 1.8	35.1 ± 1.9
Overall mean		38.0 ± 2.6[a]	17.3 ± 2.7	12.7 ± 1.2[a]	1.4 ± 0.2[b]	21.5 ± 2.5[a]	36.5 ± 4.5[a]
Fisher's LSD		1.2	1.0	0.5	0.1	0.9	1.8

[1] Values are means (n = 10 and n = 30 for overall mean) ± standard deviation.

[2] Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

[a-d] Overall means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

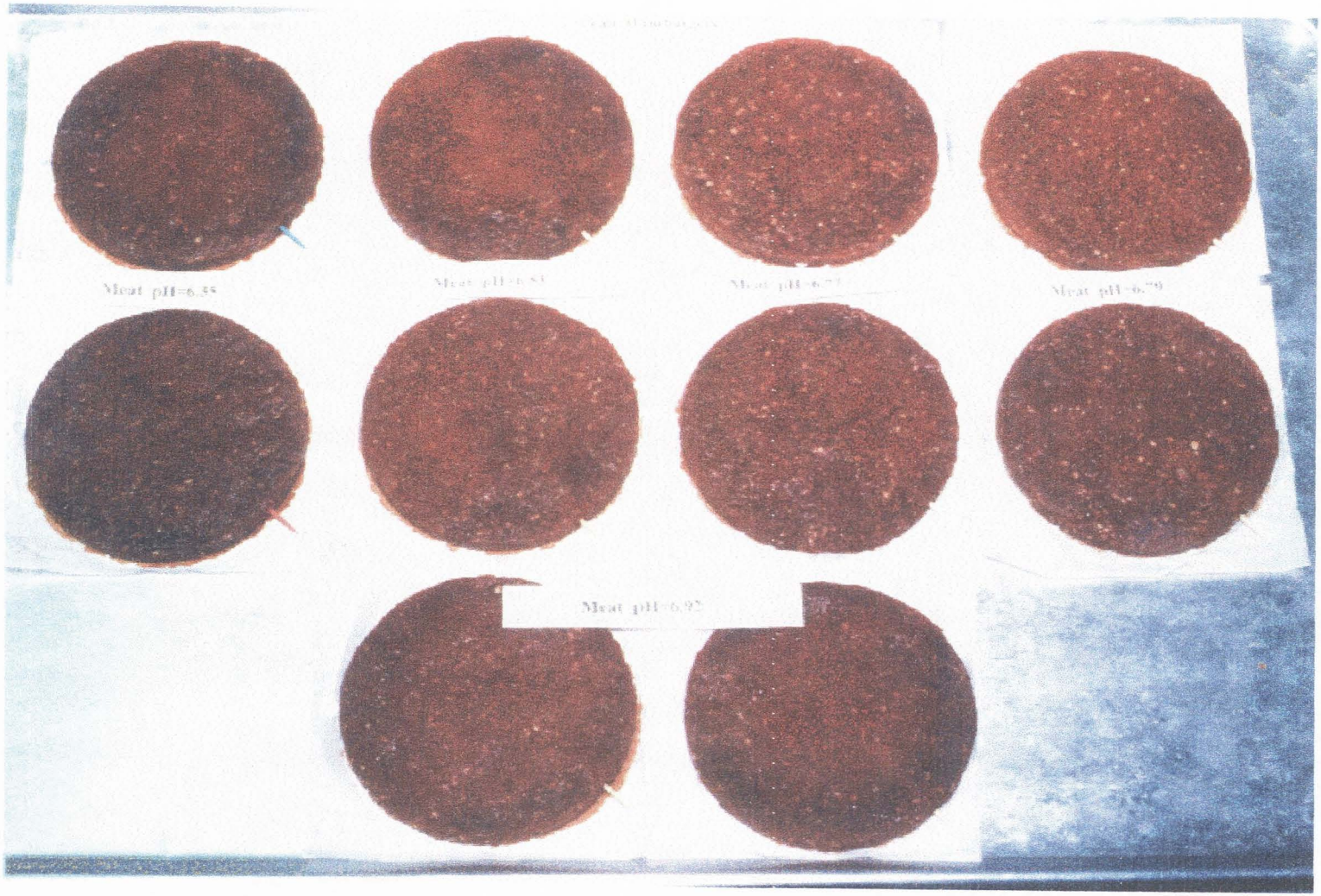

Fig. 13—Photograph of raw extra lean hamburgers of pH=6.35-6.92.

Table 15 — External Hunter color parameters of hamburgers cooked to 71°C as affected by pH and fat level

Fat level	Raw meat pH	L	a	b	a/b	Saturation index[2]	Hue angle[2]
Extra lean[1]	5.80	35.2 ± 2.5	3.3 ± 1.3	11.0 ± 1.2	0.3 ± 0.1	11.6 ± 0.9	73.0 ± 7.9
Extra lean	6.29	33.6 ± 2.9	3.1 ± 1.6	10.2 ± 1.2	0.3 ± 0.2	10.8 ± 0.8	72.3 ± 10.2
Extra lean	6.73	33.2 ± 2.8	3.4 ± 1.1	9.6 ± 1.7	0.4 ± 0.2	10.3 ± 1.3	70.0 ± 8.7
Overall mean		34.0 ± 2.8[b]	3.3 ± 1.4	10.3 ± 1.5	0.3 ± 0.2	10.9 ± 1.2	71.8 ± 8.9
Lean	5.80	39.2 ± 3.0	3.7 ± 1.3	11.3 ± 1.3	0.3 ± 0.1	12.0 ± 1.1	71.4 ± 7.2
Lean	6.29	36.5 ± 4.2	3.0 ± 0.6	10.4 ± 1.4	0.3 ± 0.1	10.9 ± 1.3	73.6 ± 4.1
Lean	6.73	36.0 ± 4.7	2.3 ± 1.4	10.5 ± 1.7	0.2 ± 0.2	10.9 ± 1.3	76.7 ± 9.7
Overall mean		37.2 ± 4.2[a]	3.0 ± 1.3	10.7 ± 1.5	0.3 ± 0.1	11.2 ± 1.3	73.9 ± 7.5
Fisher's LSD		1.9	0.7	0.6	0.1	0.5	4.2

1 Values are means (n = 15 and n = 45 for overall mean) ± standard deviation.

2 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

a-d Overall means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

The internal color of cooked hamburgers from meat of pH = 6.73 was distinctively darker, more red, and less yellow, with lowest saturation index and hue angle values, compared to burgers at low pH (Table 16; Figs. 14-16). Hunter color parameters for internal color of cooked hamburgers from meat of pH = 5.80 and pH = 6.29 were not different (Tables 88-93, Appendix D).

The internal surface of cooked lean hamburgers was lighter (L = 43.9), less red (a = 4.3), and more yellow (b = 11.0), with higher saturation index (11.9) and hue angle (68.6), than extra lean hamburgers (L = 37.1, a = 5.6, b = 9.5, saturation index = 1.3, and hue angle = 58.7, respectively; Table 17; Fig. 15). There was also a significant pH effect on internal color within the extra lean hamburger group (Table 17). Extra lean hamburgers from meat of pH = 6.73 had the highest redness (a = 7.6) and the lowest yellowness (b = 7.5), with the highest a/b ratio (1.0) and the lowest hue angle (44.9). Extra lean hamburgers from meat pH = 5.80 had the lowest redness (a = 3.9) and the highest yellowness (b = 11.3) with the lowest a/b ratio (0.3) and the highest hue angle (71.0). The internal color of hamburgers from meat pH = 6.29 was in a middle range of Hunter color values, compared to hamburgers of pH = 5.80 and 6.73.

Denaturation of myoglobin

A significantly higher amount of undenatured myoglobin (3.8 mg/g) and lower percentage of denaturation of myoglobin (47.9) was observed for extra lean hamburgers from meat of pH = 6.73, compared to pH = 5.8 (2.0 mg/g and 67.9%, respectively; Table 18; Table 95, Appendix D). Surprisingly, there were no significant

Table 16 — Internal Hunter color parameters of hamburgers cooked to 71°C as affected by pH

Raw meat pH	L	a	b	a/b	Saturation index[2]	Hue angle[2]
5.80	44.2 ± 2.6^{a}	4.1 ± 0.9^{b}	11.5 ± 0.5^{a}	0.3 ± 0.1^{b}	12.3 ± 0.5^{a}	70.5 ± 4.2^{a}
6.29	39.7 ± 6.1^{ab}	4.7 ± 1.5^{b}	10.4 ± 1.6^{a}	0.5 ± 0.2^{b}	11.6 ± 1.0^{ab}	65.1 ± 10.0^{a}
6.73	37.7 ± 6.2^{b}	6.0 ± 2.0^{a}	8.8 ± 1.7^{b}	0.7 ± 0.3^{a}	11.0 ± 0.8^{b}	55.3 ± 12.9^{b}
Fisher's LSD	4.8	1.2	1.3	0.2	0.7	8.5

1 Values are means (n = 30) ± standard deviation.

2 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{\tan-1}$.

a-d Means within column with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Fig. 14 -- Inside surface of extra lean hamburgers of pH=5.75-6.29 cooked to 71°C internal temperature.

Fig. 15 -- Inside surface of lean hamburgers of pH=6.35-6.92 (from left to right) cooked to 71°C internal temperature.

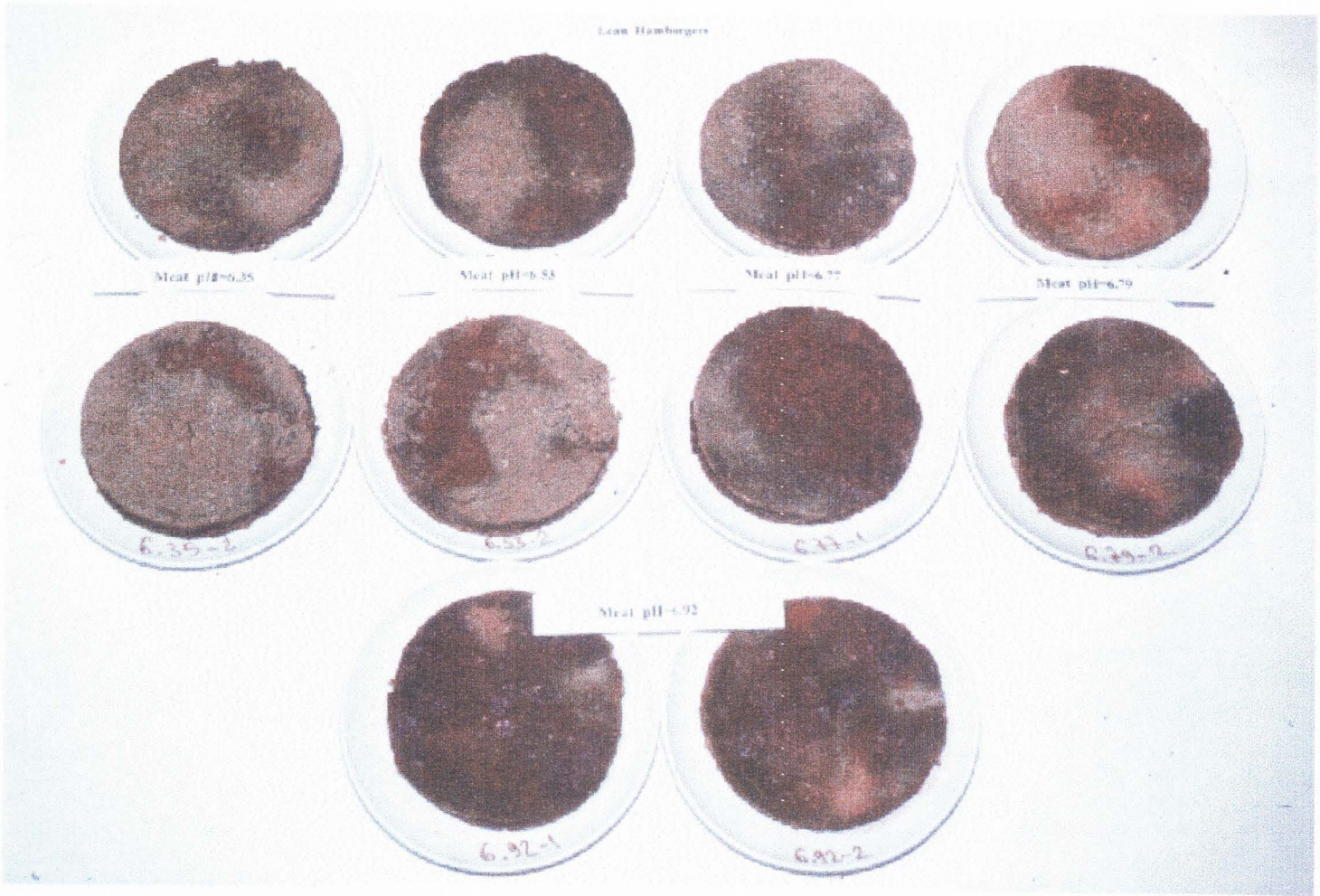

Fig. 16 -- Inside surface of extra lean hamburgers of pH=6.35-6.92 (from left to right) cooked to 71°C internal temperature.

Table 17 — Internal Hunter color parameters of hamburgers cooked to 71°C as affected by pH and fat level

Fat level	Raw meat pH	L	a	b	a/b	Saturation index[2]	Hue angle[2]
Extra lean[1]	5.80	41.85 ± 1.0	3.9 ± 1.0^{b}	11.3 ± 0.5^{a}	0.3 ± 0.1^{b}	12.0 ± 0.4	71.0 ± 4.8^{a}
Extra lean	6.29	36.4 ± 6.1	5.3 ± 1.0^{ab}	9.7 ± 1.7^{a}	0.6 ± 0.2^{b}	11.2 ± 1.2	60.3 ± 8.3^{ab}
Extra lean	6.73	33.2 ± 1.8	7.6 ± 1.0^{a}	7.5 ± 1.0^{b}	1.0 ± 0.2^{a}	10.8 ± 0.9	44.9 ± 6.1^{b}
Overall mean		37.1 ± 5.1^{b}	5.6 ± 1.8^{a}	9.5 ± 1.9^{b}	0.6 ± 0.3^{a}	11.3 ± 1.0^{b}	58.7 ± 12.6^{b}
Lean	5.80	46.5 ± 1.4	4.3 ± 0.8	11.8 ± 0.5^{a}	0.4 ± 0.1	12.5 ± 0.5	70.1 ± 3.6
Lean	6.29	43.1 ± 4.1	4.0 ± 1.6	11.1 ± 1.2^{ab}	0.4 ± 0.2	12.0 ± 0.6	69.9 ± 9.4
Lean	6.73	42.2 ± 5.6	4.5 ± 1.3	10.1 ± 1.3^{b}	0.5 ± 0.2	11.2 ± 0.7	65.7 ± 8.9
Overall mean		43.9 ± 4.4^{a}	4.3 ± 1.3^{b}	11.0 ± 1.2^{a}	0.4 ± 0.2^{b}	11.9 ± 0.8^{a}	68.6 ± 7.8^{a}
Fisher's LSD (within/between)		9.8/2.3	2.5/0.6	1.7/0.5	0.4/0.1	1.7/0.5	16.2/3.1

1 Values are means (n = 15 and n = 45 for overall mean) ± standard deviation.

2 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

a-d Means within fat level group or overall means by fat level with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 18 — Effect of pH on concentration of undenatured meat pigments in hamburgers cooked to 71°C

Raw meat pH	Cooked meat pH	Undenature dmyoglobin (mg/g)	Metmyoglobin (%)	Myoglobin denaturation (%)
5.80	6.00±0.13[c]	2.0±0.6[b]	62.8±10.5	67.9±11.8[a]
6.29	6.40±0.18[b]	3.1±0.9[ab]	59.3±5.0	59.8±9.3[a]
6.73	6.74±0.08[a]	3.8±1.3[a]	65.7±9.0	47.9±9.7[b]
Fisher's LSD	0.2	1.2	9.6	10.7

[1] Values are means (n = 20) ± standard deviation.

[a-c] Means with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

differences in myoglobin denaturation between hamburgers from meat of pH = 5.80 and pH = 6.29 (Table 97, Appendix D). The same results were obtained for redness (a) by Hunter color measurements (Table 16). The effect of pH on denaturation of myoglobin in this study is consistent with the earlier results of Trout (1989), who reported incomplete denaturation of myoglobin at temperatures lower than 76°C in cooked beef. The pH of cooked meat increased by as much as 0.2 pH units after cooking, compared with raw meat pH (Table 18; Table 94, Appendix D).

High pH had a greater protective effect myoglobin in extra lean hamburgers than in lean hamburgers at the same pH. Hamburgers of pH = 6.29 and 6.73 had

significantly higher undenaturated myoglobin concentrations and conversely a lower percentage of myoglobin denaturation than hamburgers of pH = 5.80 (Table 19). The same pattern was observed for pH effects in lean burgers. However, percentage of myoglobin denaturation was not affected by the amount of fat in hamburgers. At higher levels, beef fat affects such characteristics as thermal conductivity, density, and texture of hamburgers. Thus, high pH may be less protective for myoglobin denaturation with a higher percentage of added fat (Chapter III). Another factor that could increase the myoglobin denaturation measurement is leaching out of meat pigments with meat juice during frying of 20% fat hamburgers, which are more porous. The greatest leaching of heme pigments of beef (26% loss) occurred at 60-77°C (Buchowski et al., 1987).

Mendenhall (1989) and Trout (1989) reported that myoglobin denaturation was related to amount of total pigment or myoglobin in raw meat. In the present study, there was no significant relationship between concentration of total pigment or myoglobin and percentage of myoglobin denaturation (Table 10). However, it is understandable that at the same myoglobin denaturation rate, much more undenaturated myoglobin will be left in cooked hamburgers, if the initial total pigment was higher. With increasing initial total pigment concentration in beef, higher internal redness of cooked hamburgers will be observed. The addition of more fat to hamburgers will dilute the concentration of total pigment and will decrease internal redness of cooked hamburgers. The pH of raw meat was the most important factor affecting denaturation of myoglobin ($r = -0.72$; $p < 0.0005$), followed by oxidation-

Table 19 — Effect of pH and fat level on concentration of undenatured meat pigments in hamburgers cooked to 71°C

Fat level	Raw meat pH	Cooked meat pH	Undenatured myoglobin (mg/g)	Metmyoglobin (%)	Myoglobin denaturation (%)
Extra lean[1]	5.80	6.00±0.14^{c}	1.8±0.7^{b}	63.0±7.2	74.9±10.2^{a}
Extra lean	6.29	6.40±0.19^{b}	3.6±0.9^{ab}	57.5±2.8	55.3±11.1^{b}
Extra lean	6.73	6.80±0.08^{a}	4.4±1.3^{a}	61.2±9.7	44.2±9.6^{b}
Overall mean		6.39±0.36	3.2±1.5^{a}	60.6±7.3	58.2±16.3
Lean	5.80	6.00±0.12^{b}	2.2±0.4	62.6±13.5	60.9±9.1
Lean	6.29	6.40±0.18^{a}	2.5±0.5	61.1±6.1	64.2±3.9
Lean	6.73	6.70±0.05^{a}	3.3±1.2	70.3±5.6	51.5±8.9
Overall mean		6.37±0.31	2.7±0.9^{b}	64.6±9.7	58.9±9.2
Fisher's LSD (within/between)		0.40/0.02	1.9/0.4	16.9/5.8	18.7/6.3

[1] Values are means (n = 10) ± standard deviation.

[a-c] Means within fat level group or overall means by fat level with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

reduction potential ($r = 0.61$; $p < 0.0005$). Total reducing ability had an opposite effect on denaturation of myoglobin than reported by Warren et al. (1994). The percentage of myoglobin denaturation (PMD) increased with increasing total reducing ability. Total reducing ability cannot be used to explain premature browning or the hard-to-cook phenomena of beef patties, but total reducing ability was less in dark-cutting beef. The percentage of metmyoglobin in raw meat positively affected myoglobin denaturation but at a small extent ($r = 0.44$; $p < 0.0005$). Good correlation was observed between PMD and Hunter "a" and hue angle.

CONCLUSIONS

After freezing, grinding, and thawing, hamburgers from dark-cutting beef had attractive cherry-red color. After cooking to 71°C, the red color of undenatured myoglobin was observed inside hamburgers of high pH. At high pH, metmyoglobin formation in raw beef was reduced, and there was less denaturation of myoglobin after cooking. More severe internal dark-red color was observed in cooked hamburgers at pH = 6.73 than at pH = 6.29. The addition of extra beef fat to ground beef could be used for dilution of meat pigments and could lower internal redness in high pH hamburgers. The pH $\geq$ 6.5 and oxidation-reducing potential $\leq$ -200 mV should be used as the main characteristics of dark-cutting beef. Mixing of dark-cutting beef with normal pH ground beef may cause unequally spotted red color of high pH particles in cooked hamburgers, because myoglobin will not denature at the same rate, unless mixing is very through.

Higher cooking temperatures or food additives are needed to prevent the hard-to-cook phenomenon of hamburgers from extreme dark-cutting beef.

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CHAPTER V

PREVENTION OF PINK DISCOLORATION IN EXTRA LEAN AND LEAN DARK-CUTTING PATTIES FORMULATED WITH SALT, DEXTROSE, CARAMEL COLORANT, CALCIUM PEROXIDE, OR ENCAPSULATED LACTIC ACID

ABSTRACT

Beef patties were made from normal beef (pH = 5.70) and dark-cutting beef (pH = 6.60) at two levels of fat: extra lean (3.5%) and lean (20.0%). Controls were made with no additives or with 1% salt and 10% added water. Various browning agents (1% glucose, 0.2% caramel colorant, 0.3% calcium peroxide, or 2.5% encapsulated lactic acid) were added with 10% water and 1% salt. Dark-cutting beef (pH = 6.60) had higher concentrations of myoglobin (9.5±0.9 mg/g), metmyoglobin (26.4±1.3%), and total pigment (9.1±0.7 mg/g) than normal beef (pH = 5.70; 7.0±0.3 mg/g, 16.7±4.5%, and 6.60±0.1 mg/g, respectively). Salt had a pronounced prooxidant effect on myoglobin (Mb). The highest cooking yield (92.3%) and penetration load (1368 g) were observed for patties from dark-cutting beef. Patties of pH = 6.60 had lower shrinkage in diameter (8.8%) and higher thickness expansion (13.7%) after cooking. Extra lean patties had significantly higher initial weight and thickness expansion than lean patties. Reducing pH of meat to 5.43 by lactic acid resulted in the lowest cooked yield (81.2%) and penetration load (885 g) of all patties. Increasing meat pH to 7.03 with calcium peroxide resulted in the highest cooked yield (90.1%), penetration load (1777 g), and

lowest diameter shrinkage (8.4%) for the formulations. The external surface of patties with lactic acid was lighter (L = 40.0) and more yellow (10.9), with the highest saturation index (11.2), compared to other patties. The addition of salt to formulations significantly reduced inside surface redness and saturation index, and increased hue angle of patties in comparison with the control without any additives. Patties with encapsulated lactic acid had increased inside lightness (L = 42.7) and yellowness (b = 9.8), and significantly reduced redness (a = 2.8). A significantly higher amount of undenatured myoglobin (3.4 mg/g) and lower percentage of denaturation of myoglobin (56.8) was observed for patties from dark-cutting beef (pH = 6.60) than for normal patties (pH = 5.70; 1.7 mg/g Mb; 71.4% Mb denaturation, respectively). Distinctive absorption peaks at 541-548 nm and 577-582 nm characterized the undenatured pigment in cooked patties as oxymyoglobin. Metmyoglobin had a characteristic peak at 505 and 630 nm in cooked patty extracts. The concentration of undenatured myoglobin was 1.1 mg/g with 77.1% present as metmyoglobin form. There was 83.8% denaturation of myoglobin in cooked patties formulated with lactic acid. There were no differences in juiciness or beef flavor between dark-cutting or normal patties cooked to 71°C. However, dark-cutting patties had harder or more rubbery texture and slightly perceptible off-flavor than normal patties. Formulation with calcium peroxide caused a rubbery texture of patties, decreased juiciness, and decreased beef flavor, and had soapy off-flavor. Patties with lactic acid were less juicy and had lower intensity of beef flavor than other patties with moderate intensity of sour off-flavor. The addition of salt

and encapsulated lactic acid to beef patty formulation could solve the problem of hard-to-cook patties.

INTRODUCTION

Hard-to-cook patties, characterized by persistent internal red color during cooking, were associated with high pH raw meat, such as bull meat (Mendenhall, 1989). Undenatured myoglobin and oxymyoglobin may be present in sufficient concentration to cause red color in meats cooked to 71°C, if meat pH is greater than 6.0 (Trout, 1989; Schmidt and Trout, 1984). The authors (Mendenhall, 1989; Trout, 1989; Schmidt and Trout, 1984) suggested the reason for this phenomenon was that high pH protects myoglobin from being heat denatured. The USDA requires that beef patties be cooked to 71.1°C or until no pink color remains in the center and juices are clear (USDA, 1993; USDA-FSIS, 1993). Trout (1989) confirmed that the pH effect was the greatest at lower temperatures (55 and 62°C), where the percentage denatured myoglobin was 3 to 14 times greater at pH 5.5 than at pH of 7.0. Denaturation of meat pigments could be increased by lowering the pH or increasing the cooking temperature.

Oxidation status of myoglobin and choice of commercial beef patty formulation appeared to have an effect on the amount of myoglobin denatured by cooking to 71, 81, or 87°C internal temperature (Van Laack et al., 1996). Increasing the sodium chloride concentrations up to 3.0% increased the rate of metmyoglobin formation in raw ground beef (Trout, 1990). Sodium chloride concentrations increase the percentage of myoglobin

denaturation in beef, pork, and turkey muscle when heated to temperatures between 55 and 83°C (Trout, 1989). Salt significantly decreases the heat stability of myoglobin in the range of 68-85°C, but significantly increases the heat stability of cytochrome *c* (Ahn and Maurer, 1989).

Glycogen and glucose are depleted in dark-cutting meat. The addition of reducing sugars to beef patties from dark-cutting meat could produce brown melanoidin pigments during cooking that could reduce the pinkness in cooked meat. Brown melanoidin pigments are the products of the Maillard (nonenzymatic browning) reaction. The reaction depends on such variables as temperature, pH, moisture content, presence or absence of metal ions, and the effect of sugar structure. The products of the Maillard reaction were effective inhibitors of lipid oxidation in ground pork patties (Bedinghaus and Ockerman, 1995). However, Ahn and Maurer (1989) reported that glucose (1%) increased heat stability of hemoglobin at 68°C, and that of cytochrome *c* at 85°C.

Pink color of dark-cutting beef patties might be masked by the addition of caramel colorant. Caramel colorants are generally recognized as safe food additives and are exempt from formal FDA certification requirements. Sucrose is commonly used for making caramel colors and flavors (Fennema, 1985). The most abundant caramel colorant is acid-fast caramel made with ammonium bisulfate catalyst to produce the color for cola drinks. Caramel pigments contain hydroxyl groups of varying acidity, including carbonyl, carboxyl, enolic, and phenolic hydroxyl groups.

Historically, meat processors have used lactic acid to rapidly acidify sausages rather than rely on natural fermentation. Sausage processors cannot use uncoated lactic acid for cured meat products because it coagulates raw meat proteins and ruptures meat emulsions. Encapsulation of lactic acid by a hydrogenated vegetable oil, which will melt at 58-62°C, allows the use of lactic acid for production of cooked meat products. Approximately 100 μmol lactate per gram muscle may be produced in meat with normally low ultimate pH of 5.5 , but only 40 μmol lactate per gram would be expected in dark-cutting muscle of pH 6.2 (Tarrant and Sherington, 1980). Denaturation of meat pigments could be increased by lowering the pH of meat products (Janky and Froning, 1973). By adding of encapsulated lactic acid to dark-cutting beef, normal pH could be achieved.

Calcium peroxide has been used in dough conditioning formulations for many years (Tieckelmann and Steele, 1991). The food-grade calcium peroxide meets the Food Chemical Code requirements for use in bakery applications (FCC, 1981). About 0.25-0.5% of CaO_2 is used in flour formulations. The by-products of mixing calcium peroxide in water are lime (CaO), hydrated lime [$Ca(OH)_2$], and hydrogen peroxide (H_2O_2). The addition of CaO_2 to dark-cutting beef can oxidize and bleach the red color of myoglobin. Reaction of hydrogen peroxide with myoglobin forms peroxyl radicals that initiate lipid peroxidation in meat (Kelman et al., 1994). Many articles have been published about hydrogen peroxide related with autoxidation of oxymyoglobin and lipid oxidation (Morey et al., 1973; Tajima and Shikama, 1987).

The demand for reduced-fat beef patties is increasing because of consumer concerns regarding health and dietary fat intake. The label name "extra lean" means 10% fat or less, while "lean" or "low fat" has been defined as no more than 22% fat, by weight. When beef patties have lower than 8% fat, a reduction of tenderness, juiciness, and flavor occurs (Berry, 1992). Some properties of dark-cutting beef can be advantageous. Beef roasts from dark-cutting meat were softer, more tender, and juicier than from normal beef (Hawrysh et al., 1985). The high pH confers a greater water-holding capacity that significantly increases cooked yield.

The first objective of this study was to determine if beef patty formulations with salt, glucose, caramel color, calcium peroxide, or encapsulated lactic acid can reduce pink discoloration in dark-cutting beef patties cooked to 71°C temperature. The second objective was to evaluate sensory and physicochemical characteristics of these patties compared with normal pH beef patties.

MATERIALS AND METHODS

Patties preparation and formulation

Frozen choice inside rounds of normal and "dark-cutter" beef (pH > 6.0) were purchased from a local meat production plant. The pH of each round was measured. Rounds were classified into two pH groups (5.70 and 6.60). The external and seam fat was trimmed off. The meat from each group was mixed throughly and pH, fat, and moisture were measured before being made into patties. To make lean patties, meat was thawed at

3°C in a cooler, then ground through a 0.32-cm plate. Finely ground (0.32-cm plate) frozen beef fat was mixed in a mixer (Hollymatic Corp., Park Forest, IL) for 3 min with extra lean meat (4% fat) to adjust the fat level to 20%, then re-ground. Ground meat was spread in a thin layer on plastic trays and kept in a cooler at 3-5°C for an hour to allow meat to "bloom." Appropriate amounts of non-meat ingredients were dissolved in cold water, added to ground meat, and mixed by a dough hook in a Hobart mixer (Koch Supplies, Inc., Kansas City, MO) for 3 min. Patty formulations were: control (C2) - 1% salt and 10% tap cold water added; and C2 + 1% of D-glucose (FMC Corp., Philadelphia, PA); C2 + 0.2% caramel color (Edgar A. Weber & Company, Wheeling, IL); C2 + 0.3% CaO_2 (FMC Corp., Philadelphia, PA); C2 + 2.5% encapsulated lactic acid (lactic acid - 0.75%; CAP-SHURE® LCL-135-50, Balchem Corp., Slate Hill, NY). Beef patties were then manually formed by using a 4S 3/8 mold (Hollymatic Corp., Park Forest, IL) and separated by glassine paper. Patties from pure meat (C1) without any additives were also prepared. The meat temperature was maintained at about 5°C during grinding and forming. Wooden toothpicks (7 cm length) were inserted from the side to the geometric center of the hamburger (12 cm diameter). Toothpicks were easily removed from frozen patties for insertion of thermocouple probes. Patties were placed individually on aluminum trays and frozen in a blast freezer (-27°C) for an hour. After that, patties were tightly packaged in plastic bags, and were stored at -27 ± 5°C until needed (1- 4 months).

Moisture, fat, and pH measurements

Moisture and fat were measured as previously described in Chapter III.

Meat pigment measurements

Pigment identification was done by spectrophotometric procedures as described by Cornforth (1991). Myoglobin, metmyoglobin, and total pigment content were calculated based on absorbance of clarified extract at 525, 572, and 700 nanometers for raw and cooked meat (Trout, 1989) using a Shimadzu model UV-2100 UV-VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan). Heme pigments were extracted using 0.04 M phosphate buffer at pH 6.8 (Warriss, 1979). Myoglobin and metmyoglobin concentration was calculated using the following formula (Krzywicki, 1979):

$$\text{Myoglobin (mg / mL)} = (A_{525} - A_{700}) \text{ x } 2.303 \text{ x dilution factor} \qquad (39)$$

$$\text{Metmyoglobin (\%)} = \left(\frac{1.395 - (A_{572} - A_{700})}{(A_{525} - A_{700})} \right) \text{ x } 100 \qquad (40)$$

where A_{λ} = Absorbance at λ nm. The percentage of myoglobin denatured (PMD) was calculated using the following formula:

$$\text{PMD (\%)} = \left(1 - \frac{\text{myoglobin conc after heating}}{\text{myoglobin conc before heating}} \right) \text{ x } 100 \qquad (41)$$

Total pigment concentration was calculated using the following formula (Hornsey, 1956):

$$\text{Total pigment (ppm)} = 135.8 \text{ x } A_{640} \text{ x total vol. (mL) / sample (g)} \qquad (42)$$

where A_{640} = Absorbance at 640 nm. The factor 0.026 (Franke and Solberg, 1971) was used to convert ppm hematin to total pigment (mg/g). This factor was calculated by dividing the molecular weight of myoglobin ($\approx$ 17, 000) by the molecular weight of hematin (656) x 10^3.

Hunter color measurements

Hunter color was measured as previously described in Chapter IV.

Oxidation-reduction potential and total reducing ability measurements

Oxidation-reduction potential was measured as previously described in Chapter IV. Total reducing ability was measured as describe by Lee et al. (1981; Appendix C).

Electric grill and frying operation

Patties were fried using a Hotpoint electric grill and frying operation as previously described in Chapter III.

Physical measurements

Percentage change in hamburger thickness was ascertained as follows:

$$\Delta \text{ patty thickness } (\%) = \left(\frac{\text{Raw patty thickness} - \text{Cooked patty thickness}}{\text{Raw patty thickness}} \right) \text{x } 100 \quad (43)$$

Percentage change in hamburger diameter was determined using the follows:

$$\Delta \text{ patty diameter } (\%) = \left(\frac{\text{Raw patty diameter} - \text{Cooked patty diameter}}{\text{Raw patty diameter}} \right) \text{x } 100 \quad (44)$$

Ten patties were used for raw (at 5°C) and cooked hamburger (at 20°C) thickness and diameter measurements. Two measurements were taken per hamburger.

Cooked yield was determined on 10 patties by calculating weight differences for patties before and after cooking as follows:

$$\text{Cooking Yield (\%)} = \frac{\text{Cooked weight}}{\text{Raw weight}} \times 100 \qquad (45)$$

Penetration measurements

Penetration measurements were made was measured as previously described in Chapter III.

Trained panel sensory evaluation

Experienced meat sensory panelists (USU faculty, staff, and graduate students) were asked to participate in a training session. Six different samples were given to panelists for evaluation sensory attributes of cooked beef patties. This was followed by a group discussion where standards for each sensory attribute were established, according to research guidelines for cookery and sensory evaluations of fresh meat (AMSA, 1995). The most consistent 15 panelists were selected to be on the trained panel. The attributes evaluated were texture, juiciness, beef flavor intensity, and intensity of off-flavors. Patties were cooked to well-done (5 min total). Each cooked patty was cut into six sections, while hot. Coded sections were randomly arranged on a partitioned dinner plate (eight sections per plate) and kept covered and warm in a gas oven (about 5-10 min) before serving to the panelists. Texture, juiciness, beef flavor intensity, and intensity of off-flavors were evaluated with a 7-point structured scale, where 7 = very hard or very rubbery, very juicy, very strong beef flavor, or very intensive off-flavor and 1 = mushy, dry, no beef flavor (bland), or no detectable off-flavor (Appendix E). Taste panelists evaluated samples in partitioned booths

with red light to reduce color bias. Cold water was provided for drinking between samples. Panelists participated in six taste panel sessions with eight samples given per session

Experimental design and statistical analysis

Data were analyzed as a randomized block design with two replicates as the blocks. Treatments were arranged as a 2 x 2 x 6 factorial in a split-split plot with two pH levels: normal beef (pH = 5.70) and extreme dark-cutting beef (pH = 6.60) as the whole plot treatment; with two levels of fat: extra lean (≤ 5% fat) and lean (about 20% fat) as the subplot treatment; six patty formulations: C1 - no additives, C2 - 1% salt and 10% water added, C2 + D-glucose, C2 + caramel colorant, C2 + CaO_2, and C2 + lactic acid as the sub-subplot treatment with two or three subsamples. The study was replicated twice.

Experimental data were analyzed using the Statistical Analysis System program (Appendix G; SAS, 1988). ANOVA, GLM, and multiple comparison Fisher's LSD values ($p < 0.05$) were used for statistical analysis of physicochemical and sensory data (Tables 98-138, Appendix F).

RESULTS AND DISCUSSION

Chemical characteristics of raw meat

The compositional parameters of extra lean ground beef (initial meat source) are listed in Table 20 (Tables 98-104, Appendix F). Trimmed ground beef from inside rounds had 73.3±0.5% moisture, 3.5±1.1% fat, and 21.3±1.0% protein. Dark-cutting beef (pH = 6.60) had higher concentrations of myoglobin (9.5±0.9 mg/g),

Table 20 — Compositional characteristics of extra lean and lean ground beef (C1, no additives)

Fat level	Raw meat pH	Moisture (%)	Fat (%)	Protein[1] (%)	Myoglobin (mg/g)	Met-myoglobin (%)	Total pigment (mg/g)	ORP[2] at 24 hr (mV)	Total reducing ability
Extra lean[3]	5.70±0.02	73.7±0.4	3.5±1.2	20.9±0.9	7.0±0.3	16.7±4.5	6.6±0.1[b]	-107.6±33.8[b]	0.47±0.04[a]
Extra lean	6.60±0.02	72.8±0.1	3.5±1.0	21.8±1.0	9.5±0.9	26.4±1.3	9.1±0.7[a]	-190.9±4.6[a]	0.36±0.01[b]
Overall mean		73.3±0.5[a]	3.5±1.1[b]	21.3±1.0[a]	8.3±1.4[a]	21.6±6.0	7.8±1.4	-149.3±48.1	0.42±0.06
Lean	5.70±0.02	61.4±0.8	20.0±1.3	16.7±1.9	6.2±0.4	20.6±5.2	---	---	---
Lean	6.60±0.02	60.2±0.3	20.0±1.3	17.9±1.5	8.3±0.8	29.9±10.9	---	---	---
Overall mean		60.8±0.9[b]	20.0±1.2[a]	17.3±1.8[b]	7.2±1.3[b]	25.2±9.3	---	---	---
Fisher's LSD		2.3	1.1	3.1	0.4	18.2	0.9	34.0	0.04

1 Percentage protein was calculated by difference using typical beef composition for carbohydrates (1.2%) and inorganic salts (0.7%; Lawrie, 1968).

2 Oxidation-reduction potential values after 24 hr in ground beef chubs at 3°C.

3 Values are means (n = 6) ± standard deviation.

abc Means within groups with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

metmyoglobin (26.4±1.3%), and total pigment (9.1±0.7 mg/g) than normal beef (pH = 5.70; 7.0±0.3 mg/g, 16.7±4.5%, 6.6±0.1 mg/g, respectively). The concentration of myoglobin was the same as a total meat pigment. Meat with elevated pH had lower reducing ability values than meat with normal pH. This could be explained as a depletion of reducing agents in dark-cutting beef together with glycogen deposits. Oxidation-reduction potential (ORP) of raw dark-cutting beef was significantly lower for than normal pH meat (-191 versus -108 mV, respectively; (Table 104, Appendix F). Lower ORP of meat could stabilize myoglobin against heat denaturation and may increase pink color retention in well-cooked products (Cornforth et al., 1986). The addition of beef fat trim to extra lean meat to adjust fat level to 20% could be considered as a simple dilution factor (15-17%) that changed only the composition proportions of meat.

A separate experiment was done to check rate of metmyoglobin formation in extra lean and lean ground beef after addition of 1% salt, dissolved in 10% added water, based on meat weight. Meat myoglobin analysis was done within 2 hr after salt addition. Salt has a pronounced prooxidant effect on myoglobin. Metmyoglobin (MetMb) as percentage of total Mb significantly increased in control 2 as a result of 1% salt addition (Table 21; Table 105, Appendix F). There was no effect of meat pH or fat level on metmyoglobin formation at time of beef patty production. These results are well correlated with those of Trout (1990), who studied the effect of sodium chloride concentration (0.0-3.0%) and pH (5.5-7.0) on the metmyoglobin formation rate in ground

Table 21 — Effect of 1% salt on raw meat myoglobin and metmyoglobin concentrations in raw dark-cutting and normal ground beef

Raw meat pH	Fat level	Patty type	Myoglobin (mg/g)	Metmyoglobin (%)
5.70	Extra lean	Control 1[1]	7.0 ± 0.3	16.7 ± 4.5b
6.60	Extra lean	Control 1	9.5 ± 1.0	26.4 ± 1.3
5.70	Lean	Control 1	6.2 ± 0.4	20.6 ± 5.2
6.60	Lean	Control 1	8.3 ± 0.8	29.9 ± 10.9
Overall mean for Control 1[1]			7.7 ± 1.4[a]	23.4 ± 7.8[b]
5.70	Extra lean	Control 2[2]	6.3± 0.3	20.7 ± 5.0
6.60	Extra lean	Control 2	8.4 ± 0.8	43.8 ± 1.7
5.70	Lean	Control 2	5.2 ± 0.3	24.1 ± 3.6
6.60	Lean	Control 2	6.9 ± 0.7	47.0 ± 14.4
Overall mean for Control 2[2]			6.7 ± 1.3[b]	33.9 ± 13.9[a]
Fisher's LSD (within or between patty type)			15.8/0.1	63.6/2.6

[1-2] Control 1 - no additives; control 2 - 1% salt and 10% water added.

[a-b] Means (n = 4 and n = 16 for overall mean ± standard deviation) in columns within groups with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

beef during refrigerated storage. Increasing the salt concentration increased metmyoglobin formation; however, increasing the pH from 5.5 to 6.5 had no effect on the rate of metmyoglobin formation in ground beef. In this study, a significant difference in myoglobin concentration between control 1 and 2 could be explained by dilution of myoglobin with 10% water added to control 2 (Table 101, Appendix F).

Raw meat color

Results of Hunter color measurements of raw patties are presented in Tables 22-25. Patties from thawed dark-cutting beef had similar red color to normal patties with the same formulation (Figs, 17, 18). Dark-cutting patties were darker (L = 30.7) than normal pH patties (L = 32.0). Lean patties were lighter (L = 36.0) and more yellowish (b = 11.1) with higher saturation index (16.4) and hue angle (44.3) than extra lean patties (L = 26.6, b = 7.8, saturation index = 14.3, hue angle = 34.8, respectively; Tables 107-112, Appendix F). The hue angle may better define redness of meat than the Hunter color a-value. The larger the value for hue angle, the less red color is present in beef patties (Van Laack et al., 1996). Differences in patty formulations had a tremendous effect of raw meat color (Table 24). The caramel colorant definitely made patties look darker than the rest of the formulations. Salt reduced redness of all formulations compared with patties without additives. Dextrose seemed to stabilize red color. Calcium peroxide, caramel colorant, and lactic acid reduced redness of raw meat. Control with no additives and dextrose patty formulations had a higher color

Table 22 — Hunter color parameters of raw beef patties as affected by pH

Raw meat pH	L	a	b	a/b	Saturation index[1]	Hue angle[1]
5.70	32.0[a]	11.0	9.4	1.2	14.7	42.1
6.60	30.7[b]	12.7	9.5	1.4	16.0	37.0
Fisher's LSD	1.1	10.7	1.2	1.2	7.0	32.4

1 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

a-b Means (n = 48) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 23 — Hunter color parameters of raw beef patties as affected by fat level

Fat level	L	a	b	a/b	Saturation index[1]	Hue angle[1]
Extra lean	26.60[b]	11.9	7.8[b]	1.5[a]	14.3[b]	34.8[b]
Lean	36.0[a]	11.9	11.1[a]	1.0[b]	16.4[a]	44.3[a]
Fisher's LSD	4.2	0.9	1.1	0.03	1.6	1.4

1 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

a-b Means (n = 48) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 24 — Hunter color parameters of raw beef patties as affected by browning agents

Treatments	L	a	b	a/b	Saturation index[7]	Hue angle[7]
[1]C1	32.7[a]	16.7[a]	10.7[a]	1.6[a]	20.0[a]	32.5[d]
[1]C2	32.1[a]	13.0[b]	9.6[bc]	1.4[b]	16.2[b]	36.1[c]
[3]CaO_2	31.6[a]	9.1[c]	9.0[c]	1.0[c]	13.0[c]	46.60[a]
[4]Caramel colorant	28.4[b]	9.1[c]	8.2[d]	1.1[c]	12.2[c]	41.8[b]
[5]Dextrose	32.0[a]	14.0[b]	10.1[b]	1.4[b]	17.3[b]	35.5[cd]
[6]Lactic acid	31.1[a]	9.4[c]	9.2[c]	1.0[c]	13.3[c]	44.8[ab]
Fisher's LSD	2.5	1.5	0.6	0.2	1.4	3.5

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2+ 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[7] Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

[a-d] Means (n = 16) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 25 — Hunter color parameters of raw beef patties as affected by the interaction of pH, fat level, and browning agents

Trait	Raw meat pH	Fat level	[1]C1	[2]C2	[3]CaO_2	[4]Caramel colorant	[5]Dextrose	[6]Lactic acid
L	5.70	E. Lean	29.0^{b}	28.6^{b}	28.4^{bc}	25.1^{b}	29.5^{b}	23.6^{b}
	6.60	E. Lean	27.2^{b}	26.3^{b}	26.8^{c}	22.8^{b}	25.0^{b}	27.0^{b}
	5.70	Lean	37.5^{a}	37.4^{a}	37.2^{a}	32.1^{a}	37.4^{a}	37.5^{a}
	6.60	Lean	37.3^{a}	36.0^{a}	33.9^{ab}	33.5^{a}	36.0^{a}	36.1^{a}
a	5.70	E. Lean	17.0	14.0	6.1	9.0	15.5	9.17
	6.60	E. Lean	16.8	11.8	12.8	7.0	12.9	10.2
	5.70	Lean	15.9	11.8	5.5	8.6	13.6	6.2
	6.60	Lean	17.2	14.4	11.8	11.7	13.8	12.0
b	5.70	E. Lean	9.2^{b}	8.9^{b}	7.3^{c}	6.7^{c}	9.4^{c}	8.0^{b}
	6.60	E. Lean	8.3^{b}	7.0^{c}	8.0^{bc}	5.6^{c}	7.3^{b}	7.6^{b}
	5.70	Lean	11.8^{a}	10.6^{a}	9.6^{ab}	9.0^{b}	11.3^{a}	10.1^{a}
	6.60	Lean	13.0^{a}	11.7^{a}	11.1^{a}	11.3^{a}	12.2^{a}	11.1^{a}
a/b	5.70	E. Lean	1.8	1.6	0.8^{ab}	1.3	1.6	1.1
	6.60	E. Lean	2.0	1.7	1.6^{a}	1.2	1.8	1.3
	5.70	Lean	1.3	1.1	0.6^{b}	0.9	1.2	0.6
	6.60	Lean	1.3	1.2	1.1^{ab}	1.0	1.1	1.1
Satur. index[7]	5.70	E. Lean	19.5	16.6	9.5^{b}	11.2^{ab}	18.1	12.2
	6.60	E. Lean	18.8	13.8	15.1^{a}	9.0^{b}	14.9	12.7
	5.70	Lean	19.8	15.9	11.1^{ab}	12.5^{ab}	17.7	11.9
	6.60	Lean	21.6	18.6	16.2^{a}	16.3^{a}	18.4	16.4
Hue angle[7]	5.70	E. Lean	29.6	32.5	50.5^{ab}	36.9	31.3	41.4
	6.60	E. Lean	26.6	31.0	32.2^{b}	39.0	29.6	36.9
	5.70	Lean	36.7	42.0	60.1^{a}	46.6	39.8	58.3
	6.60	Lean	37.2	39.0	43.5^{ab}	44.6	41.5	42.8

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2 ; C2 + 1% D-glucose; C2+ 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[7] Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

[a-d] Means (n = 4) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

saturation index than other treatments. Calcium peroxide and lactic acid patty formulations had the highest hue angle values, indicating less redness of raw patties.

The interactions between pH, fat level, and patty formulation on Hunter color values of raw beef patties are presented in Table 25. Extra lean normal (pH = 5.70) patties with lactic acid had the darkest color (L = 23.6). Lean normal patties with lactic acid had the lightest color (L = 37.5). The highest redness value was for lean dark-cutting patties (pH = 6.60) without any additives (a = 13.0). The lowest redness value was observed for extra lean dark-cutting patties with caramel colorant (a = 6.1). Lean dark-cutting patties without any additives had the highest yellow color value (b = 13.0), and extra lean dark-cutting patties had the lowest yellow color (b = 5.6). The highest hue angle was observed for lean normal patties with calcium peroxide (60.1), and the lowest hue angle was observed for extra lean dark-cutting patties without any additives (26.6).

Physical properties of patties

The initial weight of patties of pH 5.70 were lower than for dark-cutting at pH 6.60 (Table 26; Table 113, Appendix F). There was no difference in cooking time (about 4:22 min). Cooking times at the same initial weight and cooking conditions was in the time range reported by Troutt et al. (1992a). The highest cooked yield (92.3%) and penetration load (1368 g) was observed for patties from dark-cutting beef. The results of the penetration test showed that with increasing pH, bind strength of patties increased significantly (Table 118, Appendix F). Different patterns of patty

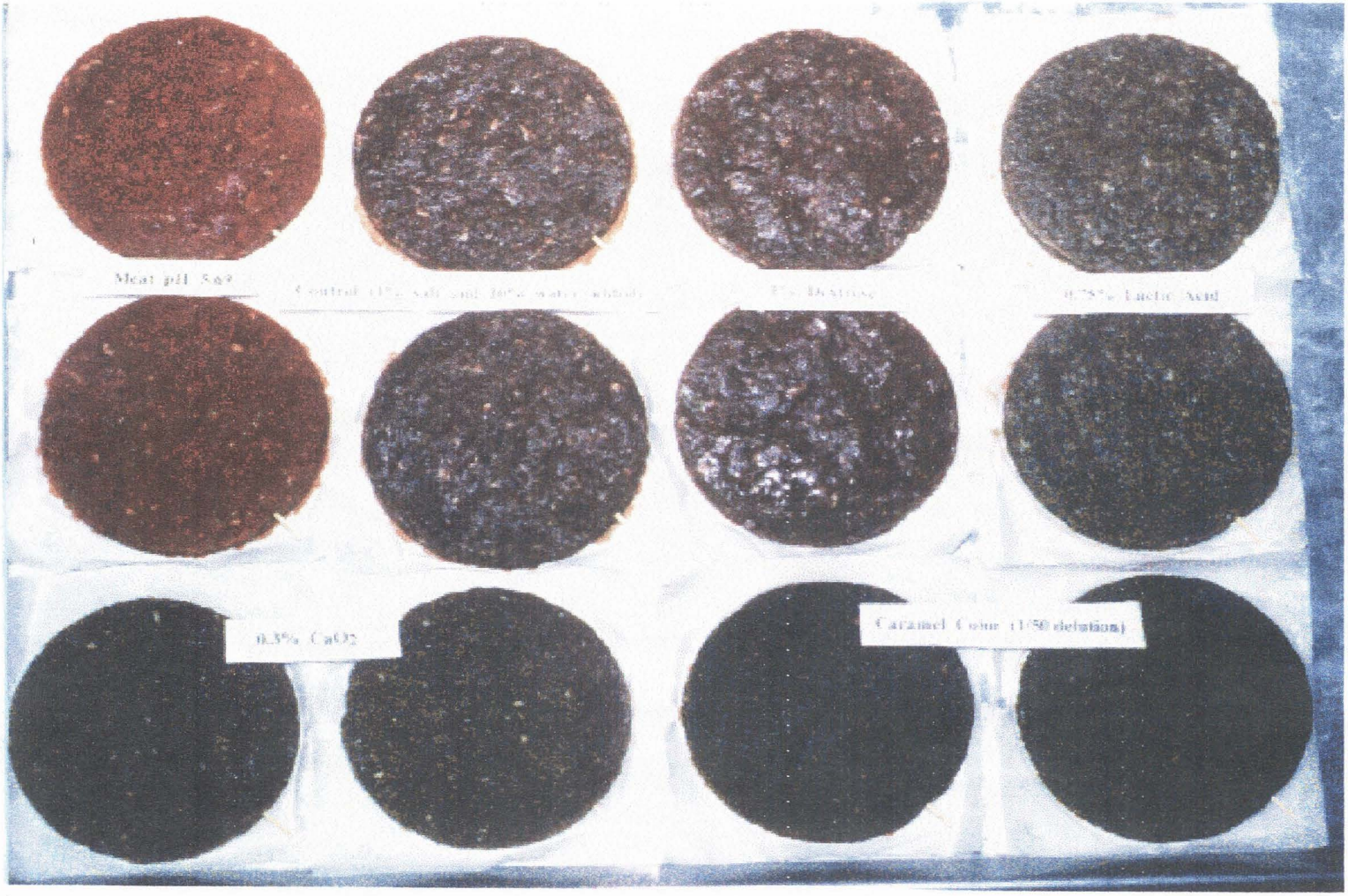

Fig. 17—Photograph of raw extra lean patties from normal beef (pH=5.70) formulated with various browning agents.

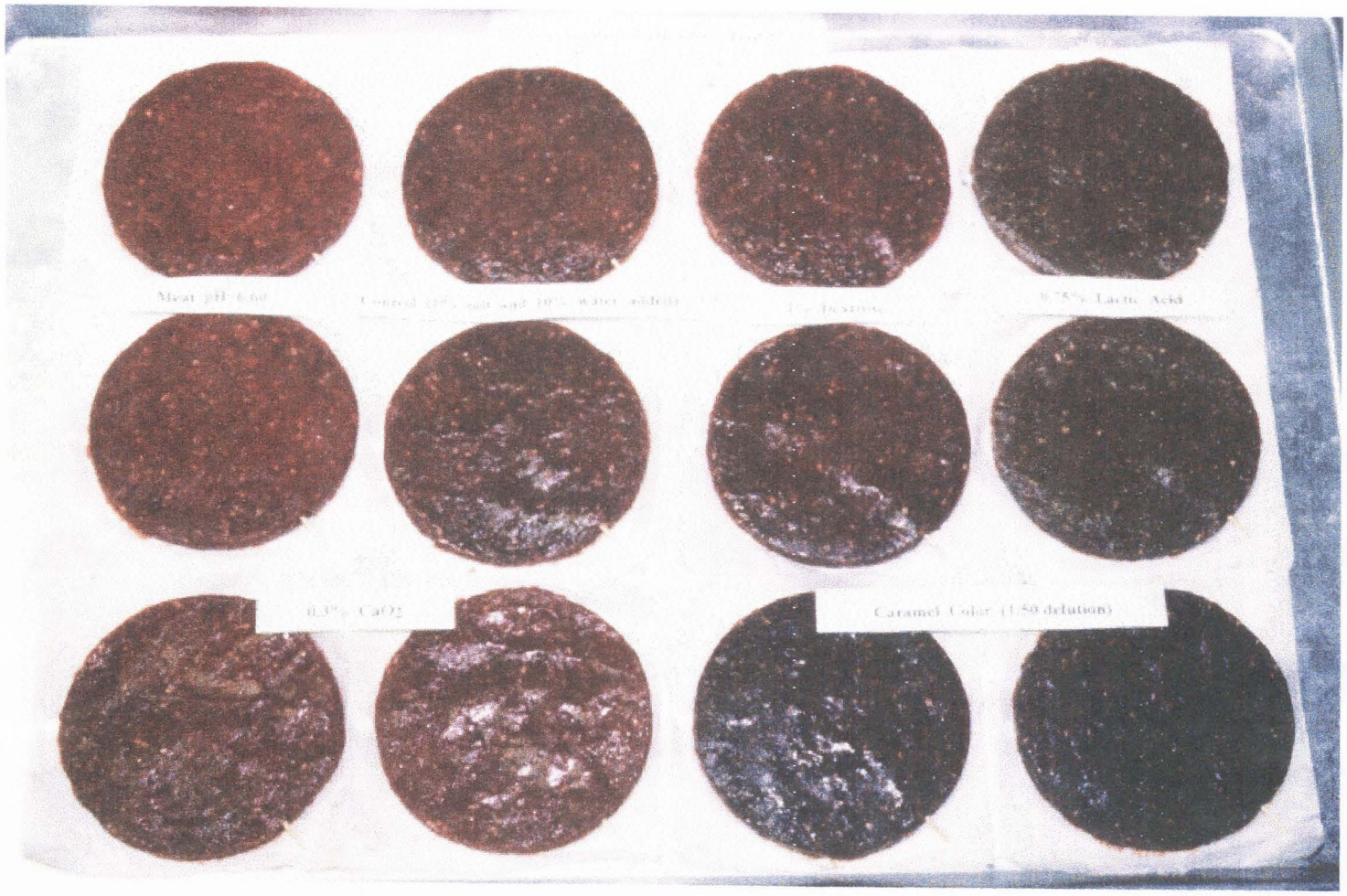

Fig. 18 – Photograph of raw extra lean patties from dark-cutting beef (pH=6.60) formulated with various browning agents. From left to right: C1 - no additives, C2 - 1% salt and 10% water added, C2 + dextrose, C2 + lactic acid, C2 + CaO_2,, and C2 + caramel colorant.

deformation during cooking were observed. Dark-cutting beef patties of pH = 6.60 had less shrinkage in diameter and higher thickness expansion after cooking than normal patties (Tables 116, 117, Appendix F).

Extra lean patties had significantly higher initial weight and thickness expansion than lean patties (Table 27; Tables 113, 117, Appendix F). Cooking time, cooked yield, diameter shrinkage, and penetration values were not significantly affected by fat level of patties (Tables 114-116, 118, Appendix F). However, the deformation pattern of patties during cooking was different. Extra lean patties had less shrinkage in diameter (9.0%) and less expansion in height (6.4%) compared to lean patties (10.5% and 12.3%, respectively). These results agree well with the higher initial weight, firmness, and deformation pattern of reduced fat-level beef patties (Troutt et al., 1992a,b; Berry, 1992)

Lactic acid and calcium peroxide patty formulations had the lowest initial weigh (Table 28). Reducing pH of meat to 5.43 with lactic acid caused lowest cooked yield and penetration load of patties. Increasing pH of meat to 7.03 with calcium peroxide resulted in patties with the highest cooked yield (90.1%), penetration load (1777 g), and lowest percentage diameter shrinkage (8.4). Addition of salt significantly increased bind strength of patties, compared to C1 without salt. Salt is known to increase cooked meat bind strength by increasing the extraction of raw meat myofibrilar proteins.

The interactions of pH, fat level, and formulation factors on physical parameters of cooked patties are presented in Table 29. Fat level, pH, and browning formulations

Table 26 — Physical characteristics of beef patties cooked to 71°C as affected by pH

Raw meat pH	Initial weight (g)	Cooking time (sec)	Yield (%)	Diameter shrinkage (%)	Thickness expansion (%)	Penetration load (g)
5.70	102.9[b]	257	84.1	10.6	5.0	825[b]
6.60	111.1[a]	267	92.3	8.8	13.7	1368[a]
Fisher's LSD	3.7	90.0	12.0	10.7	15.9	72.5

[a-b] Means (n = 48) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 27 — Physical characteristics of beef patties cooked to 71°C as affected by fat level

Fat level	Initial weight (g)	Cooking time (sec)	Yield (%)	Diameter shrinkage (%)	Thickness expansion (%)	Penetration load (g)
Extra lean	109.0[a]	260	89.0	9.0	6.4[b]	1149
Lean	105.0[b]	265	87.5	10.5	12.3[a]	1044
Fisher's LSD	1.4	16	5.70	10.7	5.70	123

[a-b] Means (n = 48) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 28 — Physical characteristics of beef patties cooked to 71°C as affected by browning agents

Treatments	Initial weight (g)	Cooking time (sec)	Yield (%)	Diameter shrinkage (%)	Thickness expansion (%)	Penetration load (g)
[1]C1	107.6[ab]	264	88.2[a]	9.3[bc]	7.5	707[d]
[1]C2	109.4[a]	266	90.1[a]	9.7[b]	10.0	1086[b]
[3]CaO_2	105.6[b]	268	90.1[a]	8.4[c]	7.5	1777[a]
[4]Caramel colorant	109.4[a]	260	90.0[a]	9.8[b]	10.6	1090[b]
[5]Dextrose	110.0[a]	253	89.8[a]	9.9[b]	11.9	1033[b]
[6]Lactic acid	100.1[c]	264	81.2[b]	11.3[a]	8.7	885[c]
Fisher's LSD	2.2	20	4.5	0.8	6.7	92

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2+ 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[a-d] Means (n = 16) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

had a significant effect on initial weight and penetration load of patties. The highest initial weight was for patties made with extra lean dark-cutting meat and dextrose (116.1 g) and the lowest weight was for lean normal patties with lactic acid (88.8 g). The highest cooked yield was for lean dark-cutting patties with calcium peroxide (95.6%) and the lowest was for lean normal patties with lactic acid (72.3%). Cooked yield of patties with added lactic acid was higher for dark-cutting patties, compared to normal meat (Table 29). The least diameter shrinkage during cooking was observed for extra lean dark-cutting patties with CaO_2 (6.8%), and the highest shrinkage was for lean normal patties with dextrose (13.1%). Lean dark-cutting patties seemed to have higher thickness expansion (up to 17.5%) than the other patties. Lowest thickness expansion was for extra lean normal patties with calcium peroxide (-2.5%). Extra lean normal patties without any additives had the lowest penetration load value (532 g), and extra lean dark-cutting patties had the highest penetration load value (2312 g).

External and internal surface color of cooked patties

Patties were cooked to 71°C internal temperature and cooking time (about 4:22 min) was the same for all patties. There was no difference between external surface Hunter color parameters of dark-cutting and normal patties, or extra lean and lean patties (Tables 30, 31; Tables 125-130, Appendix F). However, there were big differences in external color between patties of different formulations (Table 32; Figs. 19-20). The external surfaces of patties with lactic acid was lighter (L = 40.0)

Table 29 — Physical characteristics beef patties cooked to 71°C as affected by interaction of pH, fat level, and browning agents

Trait	Raw meat pH	Fat level	[1]C1	[2]C2	[3]CaO_2	[4]Caramel colorant	[5]Dextrose	[6]Lactic acid
Initial weight (g)	5.70	E. Lean	106.0^{bc}	108.0^{bc}	103.8^{b}	107.8^{b}	107.6^{bc}	96.5^{b}
	6.60	E. Lean	114.0^{a}	115.6^{a}	109.7^{a}	114.0^{a}	116.1^{a}	108.7^{a}
	5.70	Lean	102.3^{c}	102.4^{c}	102.8^{b}	105.5^{b}	103.5^{c}	88.8^{c}
	6.60	Lean	108.2^{ab}	111.8^{ab}	106.2^{ab}	110.3^{ab}	112.0^{ab}	106.4^{a}
Cooking time (sec)	5.70	E. Lean	285	275	252	258	262	264
	6.60	E. Lean	259	274	272	259	261	258
	5.70	Lean	256	270	263	266	281	272
	6.60	Lean	255	242	282	255	206	263
Yield (%)	5.70	E. Lean	84.6	86.3	93.6	86.7	86.4	77.1^{ab}
	6.60	E. Lean	94.8	96.0	82.0	95.3	95.5	89.5^{a}
	5.70	Lean	82.4	83.5	89.0	84.2	83.2	72.3^{b}
	6.60	Lean	90.9	94.7	95.6	93.5	94.2	86.0^{ab}
Diameter shrinkage (%)	5.70	E. Lean	8.5	9.5	8.5	10.4	10.2	11.0
	6.60	E. Lean	8.3	8.1	6.8	8.0	7.6	11.0
	5.70	Lean	9.7	11.8	11.2	11.6	13.1	12.0
	6.60	Lean	10.6	9.5	7.2	9.0	8.7	11.2
Thickness expansion (%)	5.70	E. Lean	0.0^{b}	0.0^{b}	-2.5	2.5	5.0	-2.5^{b}
	6.60	E. Lean	10.0^{ab}	15.0^{ab}	10.0	15.0	10.0	15.0^{a}
	5.70	Lean	2.5^{ab}	7.5^{ab}	12.5	12.5	17.5	5.0^{ab}
	6.60	Lean	17.5^{a}	17.5^{a}	10.0	12.5	15.0	17.5^{a}
Penetration load (g)	5.70	E. Lean	532^{b}	844^{c}	1464^{c}	848^{b}	714^{c}	690^{b}
	6.60	E. Lean	909^{a}	1586^{a}	2312^{a}	1296^{a}	1558^{a}	1030^{a}
	5.70	Lean	494^{b}	796^{c}	1253^{c}	802^{b}	734^{c}	726^{b}
	6.60	Lean	895^{a}	1119^{b}	2077^{b}	1413^{a}	1127^{b}	1092^{a}

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2+ 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[a-d] Means (n = 4) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 30 — External surface Hunter color parameters of beef patties cooked to 71°C as affected by pH

Raw meat pH	L	a	b	a/b	Saturation index[1]	Hue angle[1]
5.70[2]	36.7	2.9	10.8	0.3	11.3	75.0
6.60	33.2	2.6	9.0	0.3	9.5	73.4
Fisher's LSD	11.7	4.8	2.3	0.5	1.1	25.5

1 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

2 Values are means (n = 72; $p<0.05$; Fisher's least significant difference test).

Table 31 — External surface Hunter color parameters of beef patties cooked to 71°C as affected by fat level

Fat level	L	a	b	a/b	Saturation index[1]	Hue angle[1]
Extra lean[2]	33.8	2.6	9.6	0.3	10.1	74.1
Lean	36.0	2.8	10.2	0.3	10.7	74.2
Fisher's LSD	2.5	2.0	1.1	0.3	0.8	13.1

1 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

2 Values are means (n = 72; $p<0.05$; Fisher's least significant difference test).

Table 32 — External surface Hunter color parameters of beef patties cooked to 71°C as affected by browning agents

Treatments	L	a	b	a/b	Saturation index[7]	Hue angle[7]
[1]C1	35.6^{bc}	3.2^{ab}	10.4^{ab}	0.3^{ab}	11.0^{a}	72.1^{bc}
[1]C2	36.4^{b}	2.4^{bc}	9.8^{bc}	0.3^{bc}	10.2^{b}	75.9^{ab}
[3]CaO_2	31.8^{d}	1.7^{c}	9.8^{bc}	0.2^{c}	10.0^{bc}	80.3^{a}
[4]Caramel colorant	32.1^{d}	3.5^{a}	8.8^{d}	0.4^{a}	9.6^{c}	67.8^{c}
[5]Dextrose	33.7^{cd}	3.0^{ab}	9.7^{c}	0.3^{ab}	10.3^{b}	72.4^{bc}
[6]Lactic acid	40.0^{a}	2.6^{abc}	10.9^{a}	0.2^{bc}	11.2^{a}	76.3^{ab}
Fisher's LSD	1.9	1.0	0.6	0.1	0.5	6.1

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2 ; C2 + 1% D-glucose; C2+ 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[7] Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

[a-d] Means (n = 24) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

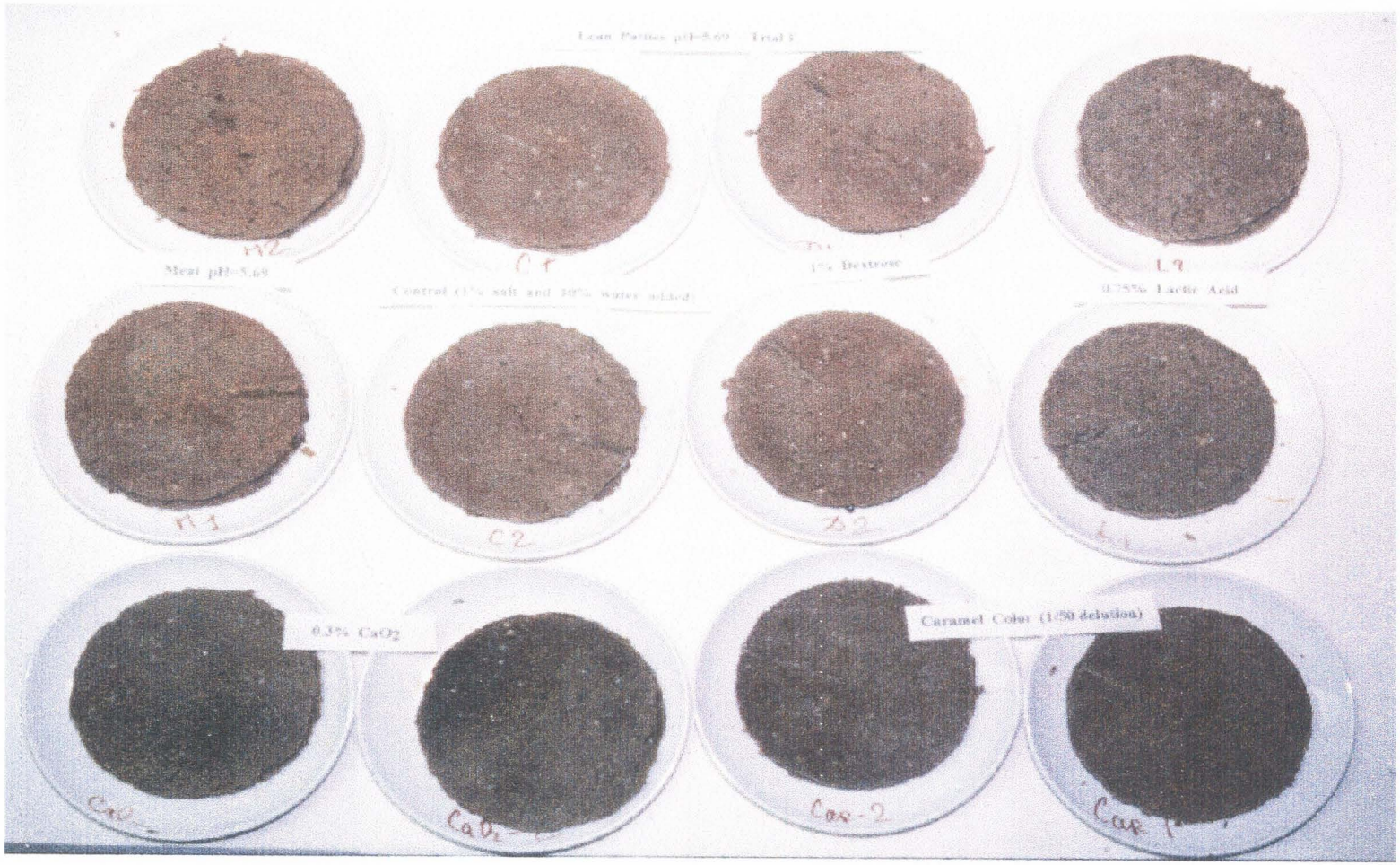

Fig. 19 -- Photograph of the internal appearance of extra lean normal patties (pH=5.70) formulated with various browning agents and cooked to 71°C internal temperature. From left to right: C1 - no additives, C2 - 1% salt and 10% water added, C2 + dextrose, C2 + lactic acid, C2 + CaO_2,, and C2 + caramel colorant.

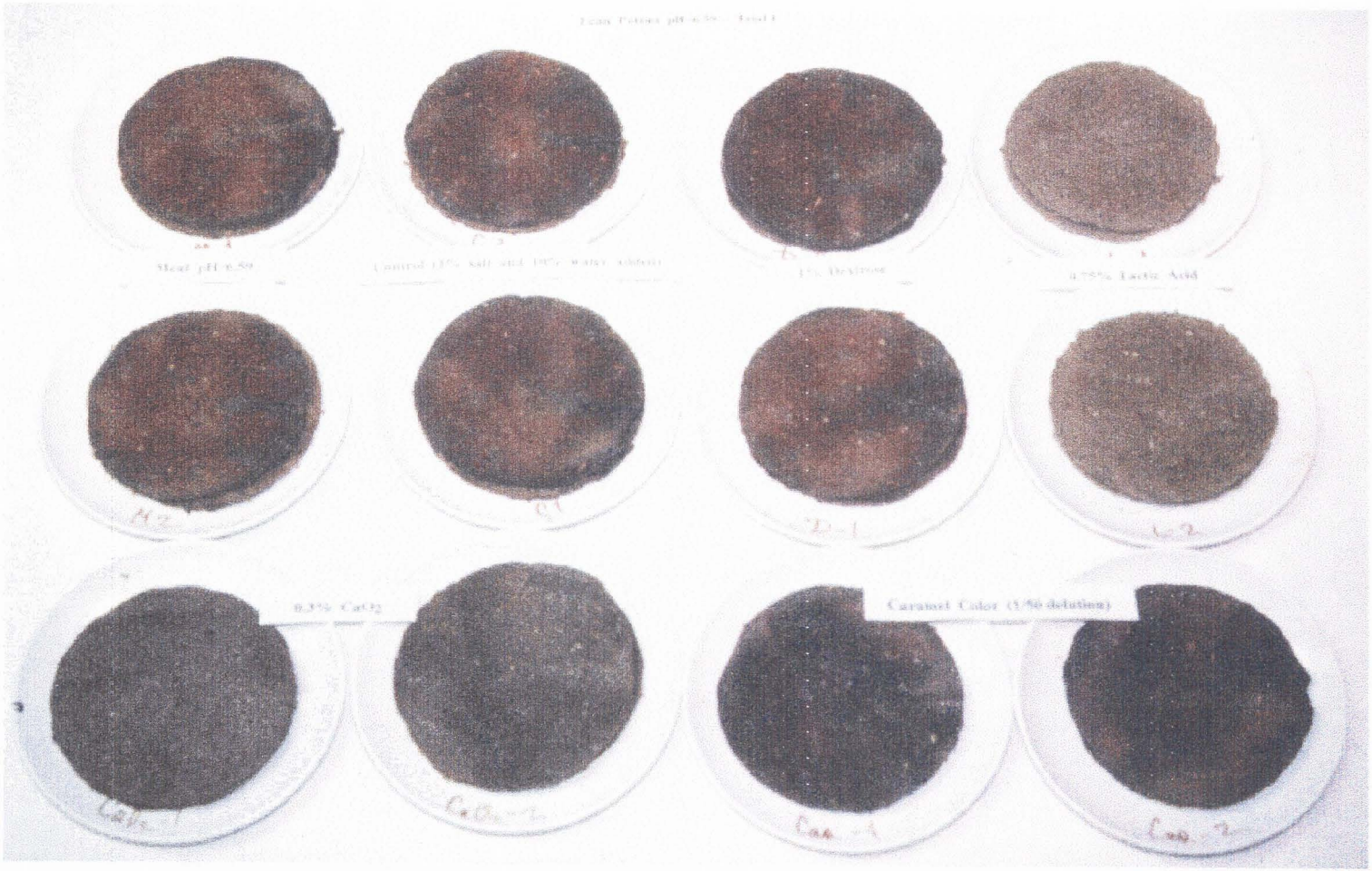

Fig. 20 -- Photograph of the internal appearance of extra lean dark-cutting patties (pH=6.60) formulated with various browning agents and cooked to 71°C internal temperature. From left to right: C1 - no additives, C2 - 1% salt and 10% water added, C2 + dextrose, C2 + lactic acid, C2 + CaO_2,, and C2 + caramel colorant.

and more yellow (10.9) with the highest saturation index (11.2), compared to the other patties. External surface of patties with caramel colorant was pinker (a = 3.5) and less yellow (b = 8.8), with lowest saturation index (9.6) and hue angle (67.8), compared to the other patties. Formulation with calcium peroxide made the external surface of patties much darker (L = 31.8) and less red (a = 1.7) with the highest hue angle (80.3). There were no significant interactions by pH, fat level, or browning agents on patty external surface color (Table 33). However, the addition of dextrose to the formulation caused the most dark-brown external surface, as observed on cooked extra lean dark-cutting patties (L = 28.9), probably due to the formation of the Maillard browning agents during cooking. The highest external surface redness (4.4) was observed for lean normal patties with dextrose, which may act as a reducing agent, identical to its stabilization of red color in raw meat (Table 24).

Surprisingly, internal Hunter color lightness and redness values of cooked patties were not significantly different between normal and dark-cutting beef patties (Table 34; Tables 125, 126, Appendix F). However, the internal color of cooked patties from normal beef was more yellow than dark-cutting patties. The internal surface of lean patties was lighter than extra lean patties (Table 35). The addition of salt to formulations significantly reduced inside surface redness and saturation index, and increased hue angle of patties in comparison to the control without any additives (Table 36; Tables 126, 129, Appendix F). Cooked patties with encapsulated lactic acid had the highest increased lightness (L = 42.7) and yellowness (b = 9.8), and

Table 33 — External surface Hunter color parameters of beef patties cooked to 71°C as affected by pH, fat level, and browning agents

Trait	Raw meat pH	Fat level	[1]C1	[2]C2	[3]CaO_2	[4]Caramel colorant	[5]Dextrose	[6]Lactic acid
L	5.70	E. Lean[7]	36.0	36.3	32.9	33.2	36.3	40.4
	6.60	E. Lean	31.9	32.2	29.3	29.9	28.9	38.4
	5.70	Lean	39.3	40.4	33.5	35.0	35.4	41.7
	6.60	Lean	35.1	36.8	31.6	30.1	34.3	39.4
a	5.70	E. Lean	2.4	2.1	0.8	4.0	2.0	2.9
	6.60	E. Lean	4.2	2.9	1.3	3.4	3.0	2.2
	5.70	Lean	4.2	2.7	3.0	2.8	4.4	2.9
	6.60	Lean	2.0	1.7	1.5	3.8	2.5	2.4
b	5.70	E. Lean	11.4	10.3	10.3	9.4	11.0	11.4
	6.60	E. Lean	8.3	8.4	8.7	7.6	7.9	10.4
	5.70	Lean	11.6	11.3	10.7	10.3	10.8	11.2
	6.60	Lean	10.2	9.1	9.5	8.0	9.0	10.5
a/b	5.70	E. Lean	0.2	0.2	0.1	0.4	0.2	0.2
	6.60	E. Lean	0.5	0.3	0.1	0.4	0.4	0.2
	5.70	Lean	0.4	0.2	0.3	0.3	0.4	0.2
	6.60	Lean	0.2	0.2	0.2	0.5	0.3	0.2
Satur. index[8]	5.70	E. Lean	11.7	10.6	10.3	10.3	11.2	11.8
	6.60	E. Lean	9.3	9.0	8.8	8.4	8.6	10.7
	5.70	Lean	12.5	11.9	11.2	10.9	11.8	11.6
	6.60	Lean	10.5	9.3	9.7	8.9	9.6	10.8
Hue angle[8]	5.70	E. Lean	77.7	77.4	85.1	66.8	79.2	75.5
	6.60	E. Lean	62.6	71.0	81.5	65.8	68.7	77.5
	5.70	Lean	69.3	76.1	73.8	74.3	67.6	75.6
	6.60	Lean	78.8	79.1	80.9	64.3	74.2	76.7

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2 + 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[7] Values are means (n = 6).

[8] Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

Table 34 — Internal Hunter color parameters of beef patties cooked to 71°C as affected by pH

Raw meat pH	L	a	b	a/b	Saturation index[1]	Hue angle[1]
5.70[2]	42.3	3.1	10.4[a]	0.3	11.0	73.9
6.60	35.0	4.6	8.1[b]	0.6	9.5	60.7
Fisher's LSD	7.4	8.3	0.3	0.6	2.1	35.8

1 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{\tan-1}$.

2 Means (n = 72) with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 35 — Internal Hunter color parameters of beef patties cooked to 71°C as affected by fat level

Fat level	L	a	b	a/b	Saturation index[1]	Hue angle[1]
Extra lean[2]	37.3[b]	4.0	9.1	0.5	10.2	65.70
Lean	40.0[a]	3.6	9.5	0.4	10.3	68.9
Fisher's LSD	1.5	1.4	1.6	0.3	0.8	11.5

1 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{\tan-1}$.

2 Means (n = 72) with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 36 — Internal Hunter color parameters of beef patties cooked to 71°C as affected by browning agents

Treatments	L	a	b	a/b	Saturation index[7]	Hue angle[7]
[1]C1	39.0^{b}	5.6^{a}	9.7^{ab}	0.6^{a}	11.5^{a}	59.1^{c}
[1]C2	38.9^{b}	4.4^{b}	9.0^{bc}	0.5^{b}	10.2^{bc}	63.1^{bc}
[3]CaO_2	38.4^{b}	1.8^{d}	9.3^{ab}	0.2^{c}	9.6^{d}	78.5^{a}
[4]Caramel colorant	34.2^{c}	4.0^{b}	8.5^{c}	0.5^{b}	9.6^{cd}	64.1^{b}
[5]Dextrose	38.8^{b}	4.2^{b}	9.3^{ab}	0.5^{b}	10.4^{b}	65.0^{b}
[6]Lactic acid	42.7^{a}	2.8^{c}	9.8^{a}	0.3^{c}	10.2^{bc}	74.1^{a}
Fisher's LSD	1.7	0.8	0.6	0.1	0.6	4.8

1-6 C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2 ; C2 + 1% D-glucose; C2 + 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

7 Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

a-d Means (n = 24) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

significantly reduced redness (a = 2.8), probably due to leaking of lactic acid out of capsules during mixing. However, the formulation with calcium peroxide had the lowest internal redness (a = 1.8), indicating that CaO_2 oxidized raw meat pigments better than the other browning treatments. Caramel colorant effectively made patties look darker (L = 34.2) and less yellow (b = 8.5), but did not affect redness. Hunter color parameters of the dextrose formulation and control 2 were not different from each other.

There were interactions of pH, fat level, and formulation factors on internal Hunter lightness and yellowness of cooked beef patties (Table 37; Tables 125, 127, Appendix F). Caramel colorant produced the most dark internal color (L = 30.8) in lean dark-cutting patties. Extra lean normal patties with lactic acid had the lightest internal color (L = 44.4). The highest redness was observed in extra lean dark-cutting patties without any additives (a = 8.2), and the lowest redness in lean normal patties with calcium peroxide (a = 1.2). The highest yellowness was observed in lean normal patties without any additives (b = 11.6), compared to the low (b = 6.6) in lean dark-cutting patties with caramel colorant.

Denaturation of myoglobin

A significantly higher amount of undenatured myoglobin (3.4 mg/g) and lower percentage of denaturation of myoglobin (56.8) were observed for cooked patties from dark-cutting beef of pH = 6.60 than for normal patties of pH = 5.70 (Table 38; Tables 131-133, Appendix F). The pH of meat increased after cooking but the difference in

Table 37 — Internal Hunter color parameters of beef patties cooked to 71°C as affected by the interaction pH, fat level, and browning agents

Trait	Raw meat pH	Fat level	[1]C1	[2]C2	[3]CaO_2	[4]Caramel colorant	[5]Dextrose	[6]Lactic acid
L	5.70	E. Lean	42.5^{ab}	42.0^{a}	39.8^{ab}	36.0^{ab}	41.8^{ab}	44.4
	6.60	E. Lean	31.5^{c}	32.8^{b}	32.9^{b}	31.7^{ab}	32.9^{c}	39.4
	5.70	Lean	45.8^{a}	43.2^{a}	43.7^{a}	38.4^{a}	44.1^{a}	45.70
	6.60	Lean	36.2^{bc}	37.4^{ab}	37.4^{ab}	30.8^{b}	$36.3b^{c}$	41.1
a	5.70	E. Lean	4.2	3.7	1.4	2.9	3.9	2.5
	6.60	E. Lean	8.2	5.5	2.2	5.1	5.2	3.4
	5.70	Lean	4.2	3.9	1.2	3.0	3.6	1.9
	6.60	Lean	6.0	4.6	2.4	4.8	4.2	3.3
b	5.70	E. Lean	11.2^{a}	10.6^{a}	10.0^{ab}	10.1^{a}	10.3^{a}	9.7
	6.60	E. Lean	7.3^{b}	7.1^{b}	7.9^{b}	7.4^{b}	7.7^{b}	9.8
	5.70	Lean	11.6^{a}	10.1^{a}	10.6^{a}	10.2^{a}	10.7^{a}	10.1
	6.60	Lean	8.7^{b}	8.3^{ab}	8.9^{ab}	6.60^{b}	8.6^{ab}	9.5
a/b	5.70	E. Lean	0.4	0.3	0.1	0.3	0.4	0.2
	6.60	E. Lean	1.1	0.8	0.3	0.7	0.7	0.3
	5.70	Lean	0.3	0.4	0.1	0.3	0.3	0.2
	6.60	Lean	0.7	0.5	0.3	0.7	0.5	0.3
Satur. index[7]	5.70	E. Lean	12.0	11.2	10.2^{a}	10.5^{a}	11.2	10.0
	6.60	E. Lean	11.0	9.1	8.2^{b}	9.0^{ab}	9.4	10.4
	5.70	Lean	12.4	11.0	10.6^{ab}	10.7^{a}	11.3	10.3
	6.60	Lean	10.5	9.5	9.2^{ab}	8.2^{b}	9.6	10.1
Hue angle[7]	5.70	E. Lean	69.4	70.7	81.6	73.9	69.2	75.4
	6.60	E. Lean	41.4	51.7	74.2	55.0	55.1	70.8
	5.70	Lean	70.1	68.9	83.5	73.3	71.7	79.4
	6.60	Lean	55.5	61.0	74.5	54.3	63.8	70.9

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2 + 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[7] Saturation index = $(a^2+b^2)^{-1/2}$; Hue angle = $(b/a)^{tan-1}$.

[a-d] Means (n = 6) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 38 — Effect of pH on denaturation of myoglobin in beef patties cooked to 71°C

Raw meat pH	pH of cooked patties	Undenatured myoglobin (mg/g)	Metmyoglobin (%)	Myoglobin denaturation (%)
5.70	6.06[b]	1.7[b]	77.3	71.4[a]
6.60	6.65[a]	3.4[a]	74.1	56.8[b]
Fisher's LSD	0.1	0.5	29.8	8.0

[a-b] Means (n = 48) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 39 — Effect of fat level on denaturation of myoglobin in beef patties cooked to 71°C

Fat level	pH of cooked patties	Undenatured myoglobin (mg/g)	Metmyoglobin (%)	Myoglobin denaturation (%)
Extra lean	6.39	2.6	72.7	66.1
Lean	6.33	2.4	78.7	62.0
Fisher's LSD	0.1	1.2	8.4	14.4

[a-b] Means (n = 48) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

pH of dark-cutting and normal beef remained (Table 131, Appendix F). The level of fat did not affect denaturation of myoglobin or pH of cooked patties (Table 39). Browning treatments had a significant effect of the denaturation of myoglobin. The effects of browning treatments and initial meat pH on myoglobin form and rate of denaturation can be clearly observed by visible absorption spectra of extracts of extra lean dark-cutting (pH = 6.60) and normal (pH = 5.70) cooked beef patties (Figs. 21-22). Distinctive absorption peaks at 541-548 nm and 577-582 nm are characteristic of undenatured oxymyoglobin. The peaks at 505 and 630 nm are characteristics of metmyoglobin (Fig.21). In the calcium peroxide sample, oxymyoglobin peaks were not observed because most of the meat pigment was oxidized to metmyoglobin, characterized by peaks at 505 and 630 mn. This observation agrees well with calculated results showing highest metmyoglobin concentration of 87.4% for the calcium peroxide formulation (Table 40). The shape and absorption of spectra maxims of cooked beef patty extracts have very good resemblance with the spectra for sperm whale skeletal muscle oxymyoglobin and metmyoglobin (after oxidation by H_2O_2; Yusa and Shikama, 1987). Hydrogen peroxide (H_2O_2) is one of the main products of calcium peroxide reaction with water. Definitely the browning agents had a higher effect on denaturation of myoglobin in normal pH meat than in dark-cutting beef. This could be explained by the higher heat stability of myoglobin at elevated pH in dark-cutting beef. Very similar visible absorption spectra were obtained by Trout (1989), who studied the relationship between meat pH, sodium chloride, sodium

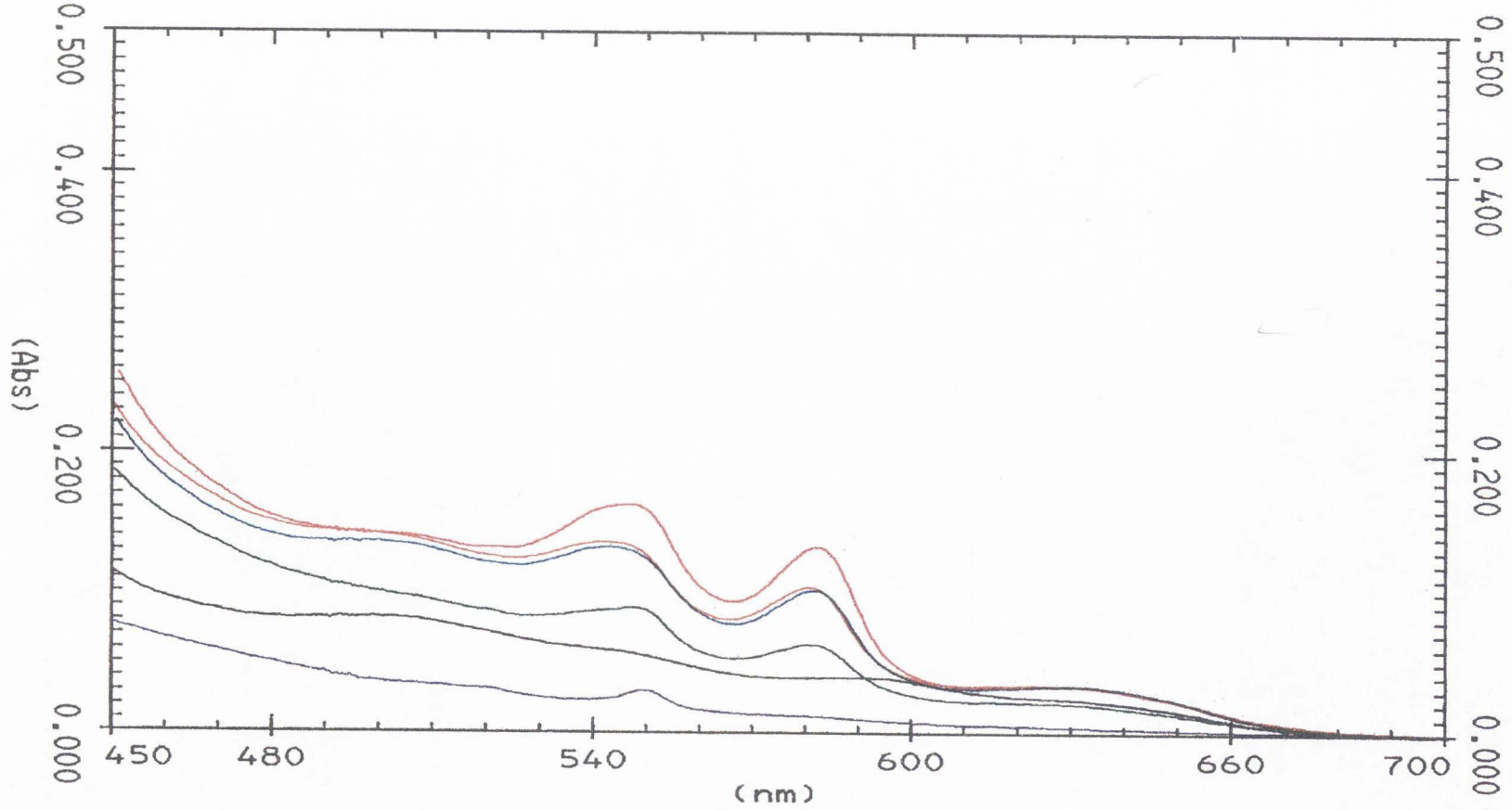

Fig. 21—Visible absorption spectra of extracts from extra lean normal patties (pH=5.70) formulated with various browning agents, and cooked to 71°C. At 480 nm from top to bottom, respectively, the curves are: C1 - no additives; C2 - 1% salt + 10% added water; caramel colorant; glucose; CaO_2; lactic acid. All browning agents were added with 1% salt and 10% water. The pigment extraction buffer was 0.04 M phosphate, pH=6.8.

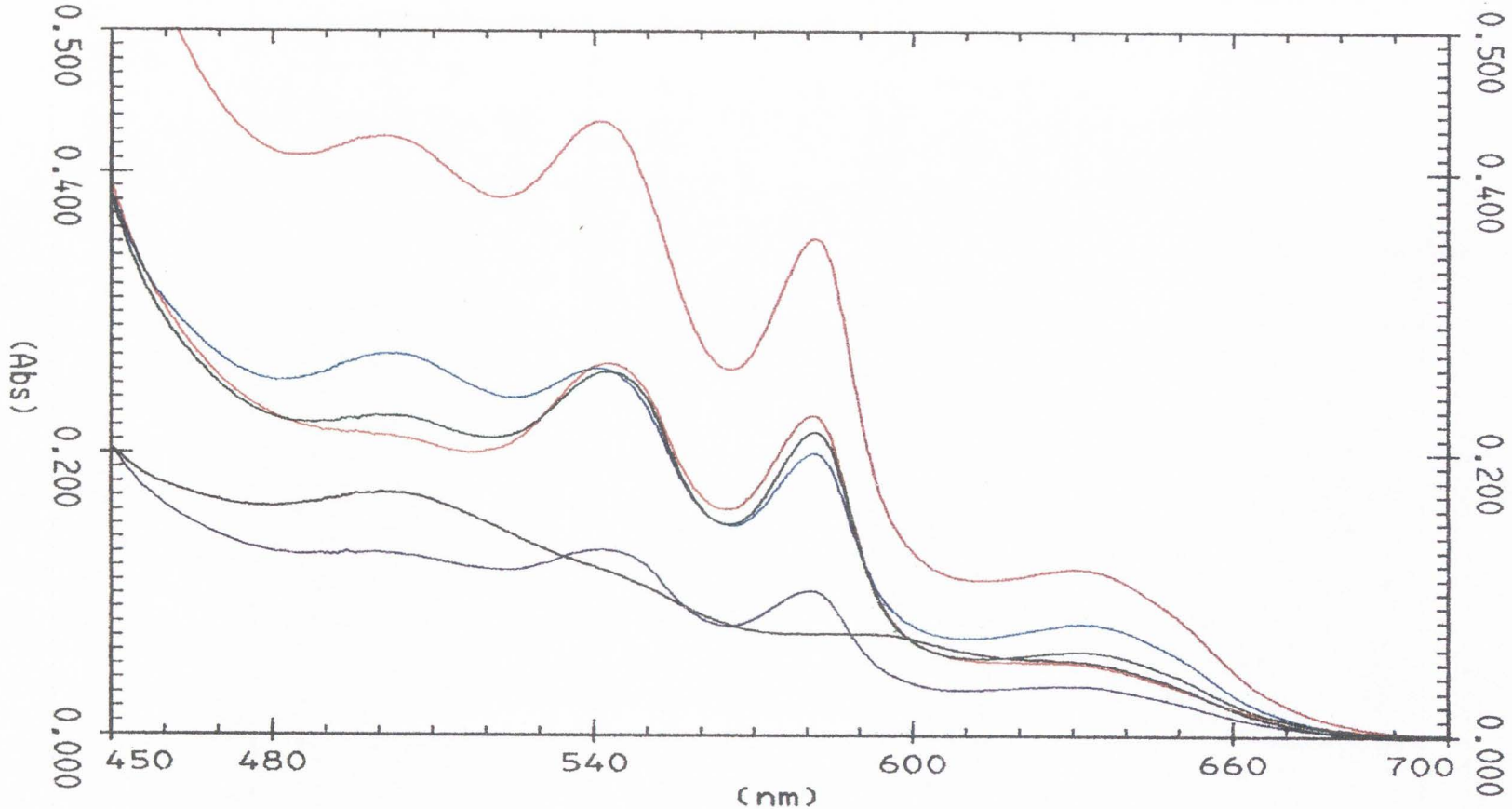

Fig. 22—Visible absorption spectra of extracts from extra lean dark-cutting patties (pH=6.60) formulated with various browning agents, and cooked to 71°C. At 480 nm from top to bottom, respectively, the curves are: C1 - no additives; C2 - 1% salt + 10% added water; caramel colorant; glucose; CaO_2; lactic acid. All browning agents were added with 1% salt and 10% water. The pigment extraction buffer was 0.04 M phosphate, pH=6.8.

Table 40 — Effect of browning agents on denaturation of myoglobin in beef patties cooked to 71°C

Treatments	pH of cooked patties	Undenatured myoglobin (mg/g)	Metmyoglobin (%)	Myoglobin denaturation (%)
[1]C1	6.37[c]	3.8[a]	69.6[d]	51.9[d]
[1]C2	6.39[c]	2.8[b]	72.2[cd]	59.1[c]
[3]CaO_2	7.03[a]	1.8[c]	87.4[a]	72.8[b]
[4]Caramel colorant	6.46[b]	2.8[b]	75.2[bc]	57.4[c]
[5]Dextrose	6.45[b]	2.7[b]	72.7[cd]	59.4[c]
[6]Lactic acid	5.43[d]	1.1[d]	77.1[b]	83.8[a]
Fisher's LSD	0.04	0.3	3.5	5.4

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2+ 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[a-d] Means (n = 16) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

tripolyphospate, and various cooking temperatures on rate of myoglobin denaturation in beef. Lactic acid had the greatest effect of all browning treatments on denaturation of myoglobin.

Lactic acid reduced meat pH to 5.43, which would favor myoglobin denaturation during cooking (Table 40). The concentration of undenatured myoglobin in cooked patties with lactic acid was 1.1 mg/g, with 77.1% of extractable pigment existing as metmyoglobin, and 83.8% denaturation of myoglobin. Calcium peroxide increased pH to 7.03 in cooked patties, lowering the percentage of myoglobin denaturation (72.8), compared with lactic acid. Also, there was a higher percentage (87.4) of metmyoglobin present in calcium peroxide treated patties, perhaps due in part to the fact that metMb is more stable to heat than either Mb or oxyMb (Janky and Froning, 1973). One percent of added salt to formulations caused lower concentrations of undenaturated myoglobin, and a higher percentage of metmyoglobin and myoglobin denaturation in comparison with cooked patties without any additives. Trout (1989) observed similar effects along with increased the pinkness of cooked meat by addition 0.5% of sodium tripolyphosphate and reduced pinkness by addition up to 3.0% of sodium chloride. The increase in pH produced by the sodium tripolyphosphate was not completely compensated by ability of tripolyphosphate ion reduce pinkness in cooked meat. Dextrose or caramel colorant did not have any effect on myoglobin denaturation in beef patties.

The highest percentage of myoglobin denaturation (91.5) was achieved at pH = 5.22 in beef patties cooked to 71°C with lactic acid added (Table 41). The lowest percentage of myoglobin denaturation (37.7) was achieved at pH = 6.72 in cooked dark-cutting beef patties without any additives. Normal meat pH patties without any additives cooked to 71°C had only 60-66% of myoglobin denaturation at pH = 6.04-6.06, which was in the range reported by Van Laack et al. (1996) for normal pH beef patties. The end cooking temperature of 71.1°C was not enough to denature 100% of myoglobin even in normal pH meat; therefore, pinkness could be present inside cooked beef patties at this cooking temperature. Internal temperatures between 81-87°C are necessary for 80.2-93.5% myoglobin denaturation in beef patties and complete disappearance of pink color (Van Laack et al., 1996). In conclusion, elevated pH markedly decreases, and low pH and oxidizing agents increase the percentage of myoglobin denaturation in beef patties at 71°C internal cooking temperature.

Trained panel sensory evaluation

There were no differences in juiciness or intensity of beef flavor between dark-cutting or normal pH = 5.70 patties cooked to 71°C (Table 42). However, dark-cutting patties had more rubbery texture and slightly intensive off-flavor, compared to normal patties. Lean patties with 20% fat had softer texture and less intense beef flavor (Table 43). The addition of fat did not affect off-flavor score but increased juiciness of beef patties. Sensory characteristics are in agreement with data of Troutt

Table 41 — Interaction effects of pH, fat level, and browning agents on denaturation of myoglobin in beef patties cooked to 71°C

Trait	Raw meat pH	Fat level	[1]C1	[2]C2	[3]CaO_2	[4]Caramel colorant	[5]Dextrose	[6]Lactic acid
pH of cooked patties	5.70	E. Lean	6.06^{b}	6.12^{b}	6.78^{c}	6.25^{b}	6.26^{b}	5.22^{b}
	6.60	E. Lean	6.72^{a}	6.69^{a}	7.17^{b}	6.74^{a}	6.74^{a}	5.89^{a}
	5.70	Lean	6.04^{b}	6.07^{b}	6.82^{c}	6.12^{b}	6.11^{b}	4.87^{c}
	6.60	Lean	6.67^{a}	6.70^{a}	7.35^{a}	6.72^{a}	6.70^{a}	5.704^{a}
Undena-tured myoglobin (mg/g)	5.70	E. Lean	2.4^{b}	1.9^{b}	1.5^{ab}	2.0^{b}	1.8^{b}	0.5
	6.60	E. Lean	5.8^{a}	3.8^{a}	2.5^{a}	3.8^{a}	3.5^{a}	1.7
	5.70	Lean	2.4^{b}	1.8^{b}	1.1^{b}	2.1^{b}	1.9^{b}	0.5
	6.60	Lean	4.7^{a}	3.6^{a}	2.1^{ab}	3.5^{a}	3.8^{a}	1.7
Met-myoglobin (%)	5.70	E. Lean	61.0	70.7	84.7	70.8	73.5	90.0^{a}
	6.60	E. Lean	70.1	64.2	89.5	72.5	64.7	60.4^{b}
	5.70	Lean	73.2	74.2	86.6	79.7	75.1	88.4^{a}
	6.60	Lean	74.2	79.7	89.0	77.7	77.5	69.4^{ab}
Myoglobin denatu-ration (%)	5.70	E. Lean	66.1^{a}	69.8^{a}	75.6	67.4^{a}	70.7^{a}	91.5
	6.60	E. Lean	37.7^{b}	53.6^{ab}	69.2	54.5^{ab}	57.6^{ab}	79.9
	5.70	Lean	60.5^{a}	65.6^{a}	78.0	58.2^{ab}	63.8^{a}	89.3
	6.60	Lean	43.2^{b}	47.2^{b}	68.6	49.6^{b}	45.4^{b}	74.6

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2 + 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[a-d] Means (n = 4) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 42 — Effect of pH on sensory scores of beef patties cooked to 71°C

Raw meat pH	Texture	Juiciness	Beef flavor	Off-flavor
5.70	4.0[b]	3.6	3.8	3.0[b]
6.60	4.5[a]	3.7	3.0	3.2[a]
Fisher's LSD	0.02	0.7	1.5	0.04

[a-b] Means (n = 363) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 43 — Effect of fat level on sensory scores of beef patties cooked to 71°C

Fat level	Texture	Juiciness	Beef flavor	Off-flavor
Extra lean	4.8[a]	3.3	3.5[a]	3.1
Lean	3.7[b]	4.0	3.3[b]	3.1
Fisher's LSD	0.1	1.1	0.1	0.5

[a-b] Means (n = 363) in columns with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

et al. (1992b) for beef patties with 5-30% fat level and Berry (1994) for ground beef patties containing 4 and 20% fat.

Formulations had a significant effect on sensory characteristics of beef patties (Table 44; Tables 135-138, Appendix F). Control patties without any additives had slightly soft texture (3.5), normal beef flavor (3.4) and were slightly juicy (2.9). The addition of salt and 10% water made patties firmer, juicier and improved beef flavor without any effect on off-flavor. Sensory characteristics of patties with dextrose and caramel colorant were not different from controls with 1% salt and 10% added water. Patties with lactic acid were less juicy (3.3) and had a low intensity of beef flavor (3.0) with moderate intensity of off-flavor (4.8). The off-flavor in lactic acid treated samples was reported by most of the taste panelists as an acid or sour flavor, which was not expected in beef patties. Formulation with calcium peroxide made texture of patties rubbery (5.3), with decreased juiciness (3.5) and beef flavor (2.4) and increased off-flavor (4.9), reported as alkaline or soapy.

Lean dark-cutting patties without any additives had the most soft texture (2.5; Table 45). A similar result was reported by Hawrysh et al. (1985) for roasts from dark-cutting beef. Extra lean dark-cutting patties with calcium peroxide had the most firm and rubbery texture (6.3). The highest sensory score for juiciness was for lean patties with caramel colorant (4.8) and the lowest juiciness score was for extra lean normal patties (2.3). The highest beef flavor score was for extra lean normal patties (4.7) with 1% salt and 10% water added, and the lowest beef flavor score was for lean dark-

Table 44 — Effect of browning agents on sensory scores of beef patties cooked to 71°C

Treatments	Texture	Juiciness	Beef flavor	Off-flavor
[1]C1	3.5^{c}	2.9^{c}	3.4^{b}	2.2^{b}
[1]C2	4.1^{b}	4.1^{a}	4.1^{a}	2.2^{b}
[3]CaO_2	5.3^{a}	3.5^{bc}	2.4^{d}	4.9^{a}
[4]Caramel colorant	4.2^{b}	4.0^{a}	3.9^{a}	2.2^{b}
[5]Dextrose	4.2^{b}	3.9^{ab}	3.8^{a}	2.2^{b}
[6]Lactic acid	4.1^{b}	3.3^{c}	3.0^{c}	4.8^{a}
Fisher's LSD	0.6	0.5	0.4	0.6

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2; C2 + 1% D-glucose; C2 + 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[a-d] Means (n = 120) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

Table 45 — Interaction effects of pH, fat level and browning agents on sensory scores of beef patties cooked to 71°C

Trait	Raw meat pH	Fat level	[1]C1	[2]C2	[3]CaO_2	[4]Caramel colorant	[5]Dextrose	[6]Lactic acid
Texture	5.70	E. Lean	4.5^{a}	4.3^{ab}	5.0^{b}	4.4^{ab}	4.2^{b}	4.7^{a}
	6.60	E. Lean	4.3^{a}	4.8^{a}	6.3^{a}	4.7^{a}	5.2^{a}	4.6^{a}
	5.70	Lean	2.6^{b}	3.4^{c}	4.2^{c}	3.5^{c}	3.0^{b}	3.7^{b}
	6.60	Lean	2.5^{b}	3.8^{bc}	5.6^{ab}	4.0^{bc}	4.6^{ab}	3.5^{b}
Juiciness	5.70	E. Lean	2.3	4.0	3.5	3.7	3.6	2.4^{b}
	6.60	E. Lean	2.9	3.6	3.0	3.6	3.5	3.4^{ab}
	5.70	Lean	3.2	4.2	3.8	4.8	4.5	3.1^{ab}
	6.60	Lean	3.3	4.7	3.8	4.0	3.8	4.4^{a}
Meat flavor	5.70	E. Lean	4.0^{a}	4.7^{a}	2.7	4.5^{a}	4.4^{a}	3.0
	6.60	E. Lean	3.3^{ab}	3.6^{b}	2.0	3.4^{b}	3.3^{b}	2.9
	5.70	Lean	3.5^{ab}	4.3^{ab}	2.6	4.5^{a}	4.2^{ab}	3.0
	6.60	Lean	2.8^{b}	3.8^{ab}	2.0	3.0^{b}	3.4^{ab}	3.0
Off-flavor	5.70	E. Lean	2.0	2.0	4.9^{ab}	1.9	2.0	5.3^{a}
	6.60	E. Lean	2.5	2.3	4.9^{ab}	2.4	2.5	4.5^{b}
	5.70	Lean	2.2	2.1	4.4^{b}	2.0	2.4	4.7^{ab}
	6.60	Lean	2.4	2.4	5.2^{a}	2.5	2.1	4.6^{ab}

[1-6] C1 - no additives; C2 - 1% salt and 10% water added; C2 + 0.3% CaO_2 ; C2 + 1% D-glucose; C2 + 0.2% caramel color; C2 + 2.5% encapsulated lactic acid, respectively.

[a-d] Means (n = 30) in columns and for the same trait with at least one common superscript letter are not significantly different ($p<0.05$; Fisher's least significant difference test).

cutting patties with calcium peroxide (2.0). The lowest off-flavor score was for extra lean normal patties with caramel colorant (1.9).

The addition of 1% salt and 10% water was beneficial for juiciness and beef flavor intensity. Dark-cutting patties had slightly intensive off-flavor. Reducing the percentage of fat caused increased firmness and beef flavor intensity. The addition of calcium peroxide also caused rubbery texture and soapy off-flavor. The addition of lactic acid made patties less juicy, reduced beef flavor, and added a sour off-flavor that may not be necessarily bad, but it is not expected in beef patties.

CONCLUSIONS

Dark-cutting beef (pH = 6.60) had higher concentrations of myoglobin metmyoglobin than normal beef (pH = 5.70). Salt (1%) had a pronounced prooxidant effect on myoglobin. The addition of salt and 10% water to formulations significantly reduced internal cooked patty redness and saturation index, and increased the hue angle value in comparison with control patties without additives. Thus salt could be effectively used to reduce mild pinkness problems in normal or dark-cutting beef patties. The elevated pH of dark-cutting patties increased cooked yield and bind strength. Significantly higher levels of undenatured myoglobin and a lower percentage of myoglobin denaturation were observed for patties from dark-cutting beef, compared with normal patties. The elevated pH of dark-cutting beef prevented myoglobin denaturation. The red internal color of cooked dark-cutting beef patties could be

reduced, but not eliminated by adding extra beef fat. Undenatured oxymyoglobin and metmyoglobin were responsible for red internal color of cooked patties. Almost total denaturation of myoglobin (84%) could be achieved by lactic acid addition to beef patties at 71°C internal temperature. The 71°C end-point cooking temperature was not sufficient for Maillard browning inside beef patties containing dextrose. However, on the external surface directly in contact with the hot grill, increased browning was observed. Surprisingly, there are no differences in juiciness or beef flavor between dark-cutting or normal patties cooked to 71°C. Dark-cutting patties had firmer and more rubbery texture, and slightly intensive off-flavor, compared to normal patties. Reducing pH by adding lactic acid resulted in almost complete myoglobin denaturation and eliminated inside pinkness in cooked, dark-cutting patties, but was also associated with a sour flavor, like in fermented sausages, that was not expected in beef patties. Calcium peroxide also reduced internal pinkness in cooked dark-cutting beef patties, but produced a very undesirable soapy off-flavor. Caramel color or dextrose formulations did not produce any off-flavors or abnormalities, but did not solve the pinkness problem inside cooked dark-cutting beef patties.

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CHAPTER VI

SUMMARY

There was no pH effect on cooking-temperature profile and cooking time for extra lean (3.3% fat) and lean hamburgers (20% fat) with different pH. The highest cooked yield and lowest cooked density were observed for hamburgers with elevated pH. Hamburgers were adequately cooked for greater than 6-log destruction of viable *E. coli* O157:H7 and *Salmonella*, except for the cold spot on the circumferential surface of hamburgers, which did not reach the required 71.1°C. The elevated pH of dark-cutting patties increased cooked yield and bind strength.

After freezing, grinding, and thawing, hamburgers from dark-cutting beef had attractive cherry-red color. After cooked to 71°C, the red color of undenatured myoglobin was observed inside hamburgers of high pH. More severe internal dark-red color was observed in cooked hamburgers at pH = 6.73 than at pH = 6.29. Significantly higher levels of undenatured myoglobin and a lower percentage of myoglobin denaturation were observed for patties from dark-cutting beef, compared with normal patties. The elevated pH of dark-cutting beef prevented myoglobin denaturation. The red internal color of cooked dark-cutting beef patties could be reduced, but not eliminated by adding extra beef fat. The pH $\geq$ 6.5 and oxidation-reducing potential $\leq$ -200 mV should be used as the main characteristics of dark-cutting beef.

Dark-cutting beef (pH = 6.60) had higher concentrations of myoglobin and total pigment than normal beef (pH = 5.70). Salt (1%) had a pronounced prooxidant effect on

myoglobin. The addition of salt to formulations significantly reduced internal cooked patty redness and saturation index, and increased the hue angle value in comparison with control patties without additives. Thus salt could be effectively used to reduce mild pinkness problems in normal or dark-cutting beef patties. Undenatured oxymyoglobin and metmyoglobin were responsible for red internal color of cooked patties. Almost total denaturation of myoglobin (84%) could be achieved by lactic acid addition to beef patties at 71°C internal temperature. The 71°C end-point cooking temperature was not sufficient for Maillard browning inside beef patties containing dextrose. Surprisingly, there are no differences in juiciness or beef flavor between dark-cutting or normal patties cooked to 71°C. Dark-cutting patties had firmer and more rubbery texture, and slightly intensive off-flavor, compared to normal patties. Caramel color or dextrose formulations did not produce any off-flavors or abnormalities, but did not solve the pinkness problem inside cooked dark-cutting beef patties. Calcium peroxide also reduced internal pinkness in cooked dark-cutting beef patties, but produced a very undesirable soapy off-flavor. Reducing pH by adding lactic acid resulted in almost complete myoglobin denaturation and eliminated inside pinkness in cooked, dark-cutting patties, but was also associated with a sour flavor, like in fermented sausages, that was not expected in beef patties.

APPENDICES

APPENDIX A.

MOISTURE AND FAT IN MEAT

Moisture measurements

Moisture was determined in raw meat for each sample. About 5 g of ground meat (sufficient to obtain about 2 g of dry material) was weighed into a pre-weighed flat-bottom aluminum dish. Meat was mixed with dried sand. Samples were weighed again. Samples were dried in a conventional oven at 100°C for 16-18 hr to a constant weight. Samples were then cooled in a desiccator for 15 min and reweighed. The percent moisture was be calculated as follows:

$$\text{Moisture (\%)} = ((W_a - W_b)/W_a) \times 100 \qquad (46)$$

where W_a = original weight meat; W_b = final weight of meat.

Fat measurements

Fat was be measured in raw meat for each sample. About 3-4 g of ground meat was be weighed into a small disposable aluminum dish, mixed with a small amount of dried sand and dried in an oven for 6 hr at 100°C. The aluminum dish was then folded and inserted into a thimble and reweighed. The thimble was placed in the condenser bracket of a Labconco Goldfish Fat Extraction Apparatus Model 35001 (Kansas City, MO) and the fat was extracted by petroleum ether for 4 hr at 100° C. After extraction and cooling in a desiccator for 15 min the thimble and contents was reweighed. Percent fat in samples was calculated as follows:

$$\text{Fat (\%)} = ((W_b - W_c)/W_a) \times 100 \qquad (47)$$

where W_a = original weight of sample; W_b = weight of thimble and contents before extraction; W_c = weight of thimble and contents after extraction.

APPENDIX B.

ANALYSIS OF VARIANCE TABLES FOR CHAPTER III

Table 46 -- Analysis of variance for moisture

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	4.00385	2.00192	0.52	0.6100
SOURCE(PH_GROUP)	12	46.61825	3.88485		
FATLEVEL	1	3450.67776	3450.67776	1123.76	0.0001
FATLEVEL*PH_GROUP	2	4.46731	2.23365	0.73	0.5033
FATLE*SOURCE(PH_GRO)	12	36.84783	3.07065		
Error	60	7.94767	0.13246		
Corrected Total	89	3550.56266			

Table 47 -- Analysis of variance for fat

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	0.87664	0.43832	0.17	0.8490
SOURCE(PH_GROUP)	12	31.68619	2.64052		
FATLEVEL	1	6303.44711	6303.44711	11550.20	0.0001
FATLEVEL*PH_GROUP	2	0.75827	0.37913	0.69	0.5182
FATLE*SOURCE(PH_GRO)	12	6.54892	0.54574		
Error	60	55.91493	0.93192		
Corrected Total	89	6399.23206			

Table 48 -- Analysis of variance for protein

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	3.395420	1.697710	0.25	0.7812
SOURCE(PH_GROUP)	12	80.798140	6.733178		
FATLEVEL	1	426.496071	426.49607	1111.87	0.0001
FATLEVEL*PH_GROUP	2	2.080216	1.040108	0.27	0.7658
FATLE*SOURCE(PH_GRO)	12	45.747913	3.812326		
Error	60	64.913200	1.081887		
Corrected Total	89	623.430960			

Table 49 -- Analysis of variance for top side starting temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	1.8923333	0.9461667	0.23	0.7956
SOURCE(PH_GROUP)	12	48.7150000	4.0595833		
FATLEVEL	1	5.9535000	5.9535000	3.38	0.0910
FATLEVEL*PH_GROUP	2	2.2230000	1.1115000	0.63	0.5490
FATLE*SOURCE(PH_GRO)	12	21.1510000	1.7625833		
Error	30	24.4950000	0.8165000		
Corrected Total	59	104.4298333			

Table 50 -- Analysis of variance for midpoint starting temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	2.25833333	1.12916667	2.47	0.1263
SOURCE(PH_GROUP)	12	5.48400000	0.45700000		
FATLEVEL	1	0.15000000	0.15000000	0.30	0.5912
FATLEVEL*PH_GROUP	2	0.46300000	0.23150000	0.47	0.6361
FATLE*SOURCE(PH_GRO)	12	5.91200000	0.49266667		
Error	30	9.1800000	0.3060000		
Corrected Total	59	23.4473333			

Table 51 -- Analysis of variance for bottom side starting temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	5.7870000	2.8935000	0.77	0.4845
SOURCE(PH_GROUP)	12	45.0790000	3.7565833		
FATLEVEL	1	12.9735000	12.9735000	2.78	0.1215
FATLEVEL*PH_GROUP	2	1.4830000	0.7415000	0.16	0.8550
FATLE*SOURCE(PH_GRO)	12	56.0710000	4.6725833		
Error	30	106.435000	3.547833		
Corrected Total	59	227.828500			

Table 52 -- Analysis of variance for top side flipping temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	172.844333	86.422167	1.43	0.2771
SOURCE(PH_GROUP)	12	724.678000	60.389833		
FATLEVEL	1	64.066667	64.066667	1.52	0.2405
FATLEVEL*PH_GROUP	2	51.442333	25.721167	0.61	0.5583
FATLE*SOURCE(PH_GRO)	12	504.226000	42.018833		
Error	30	570.90000	19.03000		
Corrected Total	59	2088.15733			

Table 53 -- Analysis of variance for midpoint flipping temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	4.644000	2.322000	0.31	0.7397
SOURCE(PH_GROUP)	12	90.105000	7.508750		
FATLEVEL	1	62.424000	62.424000	4.01	0.0684
FATLEVEL*PH_GROUP	2	129.792000	64.896000	4.17	0.0422
FATLE*SOURCE(PH_GRO)	12	186.839000	15.569917		
Error	30	443.390000	14.779667		
Corrected Total	59	917.194000			

Table 54 -- Analysis of variance for bottom side flipping temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	97.136333	48.568167	3.20	0.0770
SOURCE(PH_GROUP)	12	182.181000	15.181750		
FATLEVEL	1	24.961500	24.961500	1.24	0.2869
FATLEVEL*PH_GROUP	2	59.169000	29.584500	1.47	0.2680
FATLE*SOURCE(PH_GRO)	12	241.117000	20.093083		
Error	30	665.805000	22.193500		
Corrected Total	59	1270.369833			

Table 55 -- Analysis of variance for top side end temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	37.141000	18.570500	0.53	0.5996
SOURCE(PH_GROUP)	12	417.330000	34.777500		
FATLEVEL	1	1.040167	1.040167	0.05	0.8343
FATLEVEL*PH_GROUP	2	196.860333	98.430167	4.32	0.0386
FATLE*SOURCE(PH_GRO)	12	273.232000	22.769333		
Error	30	762.665000	25.422167		
Corrected Total	59	1688.268500			

Table 56 -- Analysis of variance for midpoint end temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	0.2643333	0.1321667	0.09	0.9145
SOURCE(PH_GROUP)	12	17.6150000	1.4679167		
FATLEVEL	1	2.2041667	2.2041667	0.85	0.3758
FATLEVEL*PH_GROUP	2	0.9143333	0.4571667	0.18	0.8412
FATLE*SOURCE(PH_GRO)	12	31.2590000	2.6049167		
Error	30	33.3250000	1.1108333		
Corrected Total	59	85.5818333			

Table 57 -- Analysis of variance for bottom side end temperature

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	0.307000	0.153500	0.01	0.9898
SOURCE(PH_GROUP)	12	178.969000	14.914083		
FATLEVEL	1	15.402667	15.402667	0.79	0.3915
FATLEVEL*PH_GROUP	2	14.994333	7.497167	0.38	0.6888
FATLE*SOURCE(PH_GRO)	12	233.893000	19.491083		
Error	30	344.610000	11.487000		
Corrected Total	59	788.176000			

Table 58 -- Analysis of variance for initial weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	635.720333	317.860167	6.48	0.0123
SOURCE(PH_GROUP)	12	588.579000	49.048250		
FATLEVEL	1	122.980167	122.980167	9.70	0.0089
FATLEVEL*PH_GROUP	2	12.740333	6.370167	0.50	0.6172
FATLE*SOURCE(PH_GRO)	12	152.107000	12.675583		
Error	30	348.87500	11.62917		
Corrected Total	59	1861.00183			

Table 59 -- Analysis of variance for initial density

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	49697.4823	24848.7412	6.48	0.0124
SOURCE(PH_GROUP)	12	46022.1620	3835.1802		
FATLEVEL	1	9621.6007	9621.6007	9.70	0.0089
FATLEVEL*PH_GROUP	2	995.4263	497.7132	0.50	0.6176
FATLE*SOURCE(PH_GRO)	12	11903.5580	991.9632		
Error	30	27279.160	909.305		
Corrected Total	59	145519.389			

Table 60 -- Analysis of variance for cooked weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	94.074333	47.037167	0.76	0.4882
SOURCE(PH_GROUP)	12	741.179000	61.764917		
FATLEVEL	1	647.473500	647.473500	33.52	0.0001
FATLEVEL*PH_GROUP	2	35.271000	17.635500	0.91	0.4275
FATLE*SOURCE(PH_GRO)	12	231.803000	19.316917		
Error	30	402.04500	13.40150		
Corrected Total	59	2151.84583			

Table 61 -- Analysis of variance for cooked density

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	53262.7341	26631.3670	5.80	0.0173
SOURCE(PH_GROUP)	12	55139.7981	4594.9832		
FATLEVEL	1	95448.7935	95448.7935	23.53	0.0004
FATLEVEL*PH_GROUP	2	3049.4935	1524.7468	0.38	0.6944
FATLE*SOURCE(PH_GRO)	12	48667.8780	4055.6565		
Error	30	89588.694	2986.290		
Corrected Total	59	345157.391			

Table 62 -- Analysis of variance for cooking time

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	6446.1000	3223.0500	1.74	0.2173
SOURCE(PH_GROUP)	12	22247.4000	1853.9500		
FATLEVEL	1	432.0167	432.0167	0.55	0.4737
FATLEVEL*PH_GROUP	2	268.6333	134.3167	0.17	0.8456
FATLE*SOURCE(PH_GRO)	12	9474.6000	789.5500		
Error	30	5216.5000	173.8833		
Corrected Total	59	44085.2500			

Table 63 -- Analysis of variance for yield

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	543.468000	271.734000	13.63	0.0008
SOURCE(PH_GROUP)	12	239.226000	19.935500		
FATLEVEL	1	164.010667	164.010667	65.33	0.0001
FATLEVEL*PH_GROUP	2	8.101333	4.050667	1.61	0.2396
FATLE*SOURCE(PH_GRO)	12	30.128000	2.510667		
Error	30	60.320000	2.010667		
Corrected Total	59	1045.254000			

Table 64 -- Analysis of variance for diameter shrinkage

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	24.860333	12.430167	1.38	0.2891
SOURCE(PH_GROUP)	12	108.203000	9.016917		
FATLEVEL	1	74.148167	74.148167	45.84	0.0001
FATLEVEL*PH_GROUP	2	0.870333	0.435167	0.27	0.7686
FATLE*SOURCE(PH_GRO)	12	19.409000	1.617417		
Error	30	22.295000	0.743167		
Corrected Total	59	249.785833			

Table 65 -- Analysis of variance for thickness expansion

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	603.33333	301.66667	3.48	0.0642
SOURCE(PH_GROUP)	12	1040.00000	86.66667		
FATLEVEL	1	735.00000	735.00000	49.00	0.0001
FATLEVEL*PH_GROUP	2	10.00000	5.00000	0.33	0.7230
FATLE*SOURCE(PH_GRO)	12	180.00000	15.00000		
Error	30	850.00000	28.33333		
Corrected Total	59	3418.33333			

Table 66 -- Analysis of variance for penetration load

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	1937851.23	968925.62	28.00	0.0001
SOURCE(PH_GROUP)	12	415278.20	34606.52		
FATLEVEL	1	146619.27	146619.27	9.09	0.0108
FATLEVEL*PH_GROUP	2	6392.63	3196.32	0.20	0.8228
FATLE*SOURCE(PH_GRO)	12	193490.60	16124.22		
Error	30	104820.00	3494.00		
Corrected Total	59	2804451.93			

APPENDIX C.

TOTAL REDUCING ABILITY OF MEAT

Total muscle reducing ability determination

1. In, duplicate, blend 2.0 g sample with 10.0 ml of 25 mM PIPES (Piperazine-n, n-bis (2-ethane-sulfonic acid) buffer using a Polytron homogenizer.
2. Transfer 5 ml of homogenate to a 10 ml volumetric flask.
3. Mix with 2 ml of 5 mM potassium ferricyanide.
4. Prepare a control of 5 ml of 25 mM PIPES and 2 ml of 5 mM potassium ferricyanide.
5. Chill at 2°C for 1 hour with occasional stirring.
6. Add 0.1 ml of 0.5% ammonium sulfamate and 0.2 ml of 0.5 M lead acetate.
7. Hold at room temperature for 5 min.
8. Add 2.5 ml of 20% trichloroacetic acid (TCA).
9. Bring solution to volume (10 ml) with distilled water.
10. After 5 min, filter through Whatman No. 42 filer paper.
11. Prepare a solution of 1 mm fericyanide.
12. Read absorbance at 420 nm of sample, control, and 1 mM ferricyanide.
13. Express total reducing ability as absorbance of 1 mM fericyanide minus absorbance of filtrate of sample plus fericyanide.

$$(1 \text{ mM ferricyanide } A_{420\ nm}) - (\text{samples } A_{420\ nm}) \qquad (48)$$

Note: Total reducing ability is an unitless value and used to evaluate the actual reducing capacity as compared to the theoretical reducing ability expressed as metmyoglobin reducing activity (MRA).

Lee, M., Cassens, R.G., and Fennema, O.R. 1981. Effect of metal ions on residual nitrite. J. Food Proc. Preserv. 5: 191-205.

APPENDIX D.

ANALYSIS OF VARIANCE TABLES FOR CHAPTER IV

Table 67 -- Analysis of variance for raw extra lean meat pH

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	8.76421000	4.38210500	53.47	0.0001
SOURCE(PH_GROUP)	12	0.98338000	0.08194833		
Error	45	0.01745000	0.00038778		
Corrected Total	59	9.76504000			

Table 68 -- Analysis of variance for raw meat myoglobin

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	13.3488033	6.6744017	1.21	0.3319
SOURCE(PH_GROUP)	12	66.1459200	5.5121600		
FATLEVEL	1	21.0633750	21.0633750	139.04	0.0001
FATLEVEL*PH_GROUP	2	0.0810300	0.0405150	0.27	0.7698
FATLE*SOURCE(PH_GRO)	12	1.8179200	0.1514933		
Error	30	1.767050	0.058902		
Corrected Total	59	104.224098			

Table 69 -- Analysis of variance for raw meat metmyoglobin

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	2880.6423	1440.3211	1.63	0.2370
SOURCE(PH_GROUP)	12	10621.4861	885.1238		
FATLEVEL	1	225.2731	225.273	13.21	0.0984
FATLEVEL*PH_GROUP	2	79.9941	39.9970	0.57	0.5802
FATLE*SOURCE(PH_GRO)	12	842.2816	70.1901		
Error	30	123.1090	4.1036		
Corrected Total	59	14772.7862			

Table 70 -- Analysis of variance for raw extra lean meat total pigment

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	2.7832467	1.3916233	0.51	0.6126
SOURCE(PH_GROUP)	12	32.7062400	2.7255200		
Error	15	0.7494500	0.0499633		
Corrected Total	29	36.2389367			

Table 71 -- Analysis of variance for raw extra lean meat oxidation-reduction potential at 24 hr

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	64182.3520	32091.1760	5.40	0.0213
Error	12	71371.8720	5947.6560		
Corrected Total	14	135554.2240			

Table 72 -- Analysis of variance for raw extra lean meat reducing ability

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	0.11736946	0.05868473	8.42	0.0052
SOURCE(PH_GROUP)	12	0.08362968	0.00696914		
Error	45	0.01519268	0.00033762		
Corrected Total	59	0.21619182			

Table 73 -- Analysis of variance for raw extra lean meat oxidation-reduction potential with time at 3°C

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TIME	4	785687.662	196421.915	25.75	0.0001
SOURCE(TIME)	20	152545.621	7627.281		
Error	44	239736.372	5448.554		
Corrected Total	68	1129617.690			

Table 74 -- Analysis of variance for raw extra lean meat myoglobin after 48 hr of storage at 3°C

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	1	26.3132042	26.3132042	11.84	0.0263
SOURCE(PH_GROUP)	4	8.8895333	2.2223833		
TIME	1	0.4620375	0.4620375	5.84	0.0731
TIME*PH_GROUP	1	0.1001042	0.1001042	1.27	0.3236
TIME*SOURCE(PH_GROU)	4	0.3165333	0.0791333		
Error	12	0.4672500	0.0389375		
Corrected Total	23	36.5486625			

Table 75 -- Analysis of variance for raw extra lean meat metmyoglobin after 48 hr of storage at 3°C

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	1	568.52400	568.52400	1.46	0.2936
SOURCE(PH_GROUP)	4	1558.34753	389.58688		
TIME	1	405.82150	405.82150	60.72	0.0015
TIME*PH_GROUP	1	469.84650	469.84650	70.30	0.0011
TIME*SOURCE(PH_GROU)	4	26.73497	6.68374		
Error	12	73.29855	6.10821		
Corrected Total	23	3102.57306			

Table 76 -- Analysis of variance for Hunter color "L" of raw hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	12.56033	6.28017	0.47	0.6356
SOURCE(PH_GROUP)	12	160.08900	13.34075		
FATLEVEL	1	1339.53750	1339.53750	292.14	0.0001
FATLEVEL*PH_GROUP	2	6.44700	3.22350	0.70	0.5144
FATLE*SOURCE(PH_GRO)	12	55.02300	4.58525		
Error	30	7.78500	0.25950		
Corrected Total	59	1581.44183			

Table 77 -- Analysis of variance for Hunter color "a" of raw hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	186.636000	93.318000	4.64	0.0322
SOURCE(PH_GROUP)	12	241.350000	20.112500		
FATLEVEL	1	5.104167	5.104167	1.63	0.2260
FATLEVEL*PH_GROUP	2	1.409333	0.704667	0.22	0.8019
FATLE*SOURCE(PH_GRO)	12	37.594000	3.132833		
Error	30	19.335000	0.644500		
Corrected Total	59	491.428500			

Table 78 -- Analysis of variance for Hunter color "b" of raw hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	2.905333	1.452667	0.31	0.7375
SOURCE(PH_GROUP)	12	55.808000	4.650667		
FATLEVEL	1	178.882667	178.882667	247.99	0.0001
FATLEVEL*PH_GROUP	2	3.561333	1.780667	2.47	0.1265
FATLE*SOURCE(PH_GRO)	12	8.656000	0.721333		
Error	30	2.400000	0.080000		
Corrected Total	59	252.213333			

Table 79 -- Analysis of variance for Hunter color "a/b" ratio of raw hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	1.19329000	0.59664500	6.82	0.0105
SOURCE(PH_GROUP)	12	1.05002000	0.08750167		
FATLEVEL	1	2.93488167	2.93488167	161.09	0.0001
FATLEVEL*PH_GROUP	2	0.10302333	0.05151167	2.83	0.0986
FATLE*SOURCE(PH_GRO)	12	0.21862000	0.01821833		
Error	30	0.41045000	0.01368167		
Corrected Total	59	5.91028500			

Table 80 -- Analysis of variance for Hunter color saturation index of raw hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	146.181173	73.090587	3.43	0.0662
SOURCE(PH_GROUP)	12	255.447350	21.287279		
FATLEVEL	1	84.942202	84.942202	33.60	0.0001
FATLEVEL*PH_GROUP	2	1.667613	0.833807	0.33	0.7254
FATLE*SOURCE(PH_GRO)	12	30.336710	2.528059		
Error	30	10.079650	0.335988		
Corrected Total	59	528.654698			

Table 81 -- Analysis of variance for Hunter color hue angle of raw hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	366.056623	183.028312	5.86	0.0168
SOURCE(PH_GROUP)	12	374.974900	31.247908		
FATLEVEL	1	789.525375	789.525375	79.38	0.0001
FATLEVEL*PH_GROUP	2	12.376290	6.188145	0.62	0.5532
FATLE*SOURCE(PH_GRO)	12	119.360160	9.946680		
Error	30	102.58215	3.41941		
Corrected Total	59	1764.87550			

Table 82 -- Analysis of variance for external Hunter color "L" of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	119.014889	59.507444	1.99	0.1789
SOURCE(PH_GROUP)	12	358.331333	29.860944		
FATLEVEL	1	227.529000	227.529000	13.79	0.0030
FATLEVEL*PH_GROUP	2	6.782000	3.391000	0.21	0.8170
FATLE*SOURCE(PH_GRO)	12	198.000667	16.500056		
Error	60	437.833333	7.297222		
Corrected Total	89	1347.491222			

Table 83 -- Analysis of variance for external Hunter color "a" of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	7.2246667	3.6123333	0.70	0.5156
SOURCE(PH_GROUP)	12	61.8886667	5.1573889		
FATLEVEL	1	1.3201111	1.3201111	0.58	0.4592
FATLEVEL*PH_GROUP	2	8.3908889	4.1954444	1.86	0.1981
FATLE*SOURCE(PH_GRO)	12	27.0873333	2.2572778		
Error	60	52.593333	0.876556		
Corrected Total	89	158.505000			

Table 84 -- Analysis of variance for external Hunter color "b" of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	19.3786667	9.6893333	1.43	0.2769
SOURCE(PH_GROUP)	12	81.2113333	6.7676111		
FATLEVEL	1	4.8534444	4.8534444	2.52	0.1382
FATLEVEL*PH_GROUP	2	2.2675556	1.1337778	0.59	0.5699
FATLE*SOURCE(PH_GRO)	12	23.0806667	1.9233889		
Error	60	69.433333	1.157222		
Corrected Total	89	200.225000			

Table 85 -- Analysis of variance for external Hunter color "a/b" of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	0.00446889	0.00223444	0.03	0.9736
SOURCE(PH_GROUP)	12	0.99959333	0.08329944		
FATLEVEL	1	0.03969000	0.03969000	1.27	0.2822
FATLEVEL*PH_GROUP	2	0.09632667	0.04816333	1.54	0.2542
FATLE*SOURCE(PH_GRO)	12	0.37563333	0.03130278		
Error	60	0.85226667	0.01420444		
Corrected Total	89	2.36797889			

Table 86 -- Analysis of variance for external Hunter color saturation index of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	23.5456867	11.7728433	2.59	0.1158
SOURCE(PH_GROUP)	12	54.4659867	4.5388322		
FATLEVEL	1	2.7352900	2.7352900	1.99	0.1834
FATLEVEL*PH_GROUP	2	1.1845400	0.5922700	0.43	0.6592
FATLE*SOURCE(PH_GRO)	12	16.4687200	1.3723933		
Error	60	43.9984667	0.7333078		
Corrected Total	89	142.3986900			

Table 87 -- Analysis of variance for external Hunter color hue angle of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	18.61452	9.30726	0.04	0.9577
SOURCE(PH_GROUP)	12	2576.75951	214.72996		
FATLEVEL	1	97.92727	97.92727	1.17	0.3007
FATLEVEL*PH_GROUP	2	270.06998	135.03499	1.61	0.2396
FATLE*SOURCE(PH_GRO)	12	1004.56075	83.71340		
Error	60	2118.92513	35.31542		
Corrected Total	89	6086.85716			

Table 88 -- Analysis of variance for internal Hunter color "L" of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	656.15622	328.07811	4.40	0.0370
SOURCE(PH_GROUP)	12	895.75667	74.64639		
FATLEVEL	1	1040.40000	1040.40000	40.43	0.0001
FATLEVEL*PH_GROUP	2	73.37400	36.68700	1.43	0.2783
FATLE*SOURCE(PH_GRO)	12	308.80600	25.73383		
Error	60	79.10667	1.31844		
Corrected Total	89	3053.59956			

Table 89 -- Analysis of variance for internal Hunter color "a" of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	59.6535556	29.8267778	6.08	0.0150
SOURCE(PH_GROUP)	12	58.8453333	4.9037778		
FATLEVEL	1	42.1617778	42.1617778	22.99	0.0004
FATLEVEL*PH_GROUP	2	44.8962222	22.4481111	12.24	0.0013
FATLE*SOURCE(PH_GRO)	12	22.0053333	1.8337778		
Error	60	32.500000	0.541667		
Corrected Total	89	260.062222			

Table 90 -- Analysis of variance for internal Hunter color "b" of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	110.573556	55.286778	10.36	0.0024
SOURCE(PH_GROUP)	12	64.012667	5.334389		
FATLEVEL	1	48.107111	48.107111	33.30	0.0001
FATLEVEL*PH_GROUP	2	16.557556	8.278778	5.73	0.0179
FATLE*SOURCE(PH_GRO)	12	17.335333	1.444611		
Error	60	22.640000	0.377333		
Corrected Total	89	279.226222			

Table 91 -- Analysis of variance for internal Hunter color "a/b" ratio of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	2.35907556	1.17953778	8.23	0.0056
SOURCE(PH_GROUP)	12	1.71908000	0.14325667		
FATLEVEL	1	1.40125444	1.40125444	59.45	0.0001
FATLEVEL*PH_GROUP	2	1.27259556	0.63629778	26.99	0.0001
FATLE*SOURCE(PH_GRO)	12	0.28286667	0.02357222		
Error	60	0.80266667	0.01337778		
Corrected Total	89	7.83753889			

Table 92 -- Analysis of variance for internal Hunter color saturation index of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	25.2763800	12.6381900	7.58	0.0074
SOURCE(PH_GROUP)	12	20.0135467	1.6677956		
FATLEVEL	1	7.1177344	7.1177344	5.49	0.0372
FATLEVEL*PH_GROUP	2	0.5573489	0.2786744	0.21	0.8097
FATLE*SOURCE(PH_GRO)	12	15.5616667	1.2968056		
Error	60	14.8701333	0.2478356		
Corrected Total	89	83.3968100			

Table 93 -- Analysis of variance for internal Hunter color hue angle of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	3575.16931	1787.58465	7.73	0.0070
SOURCE(PH_GROUP)	12	2774.10038	231.17503		
FATLEVEL	1	2169.72900	2169.72900	46.56	0.0001
FATLEVEL*PH_GROUP	2	1754.29194	877.14597	18.82	0.0002
FATLE*SOURCE(PH_GRO)	12	559.21146	46.60096		
Error	60	992.0712	16.5345		
Corrected Total	89	11824.5733			

Table 94 -- Analysis of variance for pH of cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	11.3123850	5.6561925	33.29	0.0001
SOURCE(PH_GROUP)	12	2.0388900	0.1699075		
FATLEVEL	1	0.0158700	0.0158700	4.04	0.0675
FATLEVEL*PH_GROUP	2	0.0590450	0.0295225	7.51	0.0077
FATLE*SOURCE(PH_GRO)	12	0.0471600	0.0039300		
Error	90	0.0490500	0.0005450		
Corrected Total	119	13.5224000			

Table 95 -- Analysis of variance for undenatured myoglobin in cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	33.3300700	16.6650350	5.30	0.0224
SOURCE(PH_GROUP)	12	37.7150200	3.1429183		
FATLEVEL	1	4.8735000	4.8735000	9.77	0.0088
FATLEVEL*PH_GROUP	2	8.3001100	4.1500550	8.32	0.0054
FATLE*SOURCE(PH_GRO)	12	5.9878400	0.4989867		
Error	30	0.4178000	0.0139267		
Corrected Total	59	90.6243400			

Table 96 -- Analysis of variance for undenatured metmyoglobin in cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	417.55800	208.77900	1.08	0.3702
SOURCE(PH_GROUP)	12	2317.95002	193.16250		
FATLEVEL	1	249.36971	249.36971	2.31	0.1546
FATLEVEL*PH_GROUP	2	226.93630	113.46815	1.05	0.3800
FATLE*SOURCE(PH_GRO)	12	1296.75124	108.06260		
Error	30	41.32130	1.37738		
Corrected Total	59	4549.88657			

Table 97 -- Analysis of variance for percent myoglobin denaturation in cooked hamburgers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_GROUP	2	4073.77561	2036.88781	8.43	0.0052
SOURCE(PH_GROUP)	12	2898.09494	241.50791		
FATLEVEL	1	7.58282	7.58282	0.06	0.8097
FATLEVEL*PH_GROUP	2	1631.47339	815.73669	6.52	0.0121
FATLE*SOURCE(PH_GRO)	12	1501.16072	125.09673		
Error	30	83.2572	2.7752		
Corrected Total	59	10195.3447			

APPENDIX E.

SENSORY BALLOT FOR TRAINED TASTE PANEL

Evaluation Sheet for Trained Panel
PRODUCT: COOKED BEEF PATTIES

Name _____ Date _____

Please sample in the order below. Use the following scale for evaluation the sample characteristics. Note: you can use any of the numbers regardless of whether defined.

1. Texture

7 Very hard
6
5 Hard
4
3 Slightly soft
2
1 Mushy

2. Juiciness

7 Very juicy
6
5 Moderate juicy
4
3 Slightly juicy
2
1 Dry

3. Beef/Meat Flavor

7 Strong flavor
6
5 Moderate flavor
4
3 Slight flavor
2
1 No flavor (bland)

4. Off-Flavors

7 Very intensive
6
5 Moderate intensive
4
3 Slightly intensive
2
1 Not detectable

Sample #	*Texture*	*Juiciness*	*Beef Flavor*	*Off-Flavor*	*Comments*
770					
247					
848					
114					
356					
563					

APPENDIX F.

ANALYSIS OF VARIANCE TABLES FOR CHAPTER V

Table 98 -- Analysis of variance for raw patties moisture

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_TYPE	1	6.437704	6.437704	22.38	0.0419
REP(PH_TYPE)	2	0.575242	0.287621		
FATLEVEL	1	939.125704	939.125704	525.97	0.0019
FATLEVEL*PH_TYPE	1	0.189037	0.189037	0.11	0.7758
FATLEVE*REP(PH_TYPE)	2	3.571008	1.785504		
Error	16	0.589800	0.036863		
Corrected Total	23	950.488496			

Table 99 -- Analysis of variance for raw patties fat

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_TYPE	1	0.00070	0.00070	0.00	0.9931
REP(PH_TYPE)	2	14.90651	7.45325		
FATLEVEL	1	1636.96684	1636.96684	4065.78	0.0002
FATLEVEL*PH_TYPE	1	0.00350	0.00350	0.01	0.9342
FATLEVE*REP(PH_TYPE)	2	0.80524	0.40262		
Error	16	14.14660	0.88416		
Corrected Total	23	1666.82940			

Table 100 -- Analysis of variance for raw patties protein

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_TYPE	1	6.5730667	6.5730667	0.62	0.5130
REP(PH_TYPE)	2	21.1426667	10.5713333		
FATLEVEL	1	96.3202667	96.3202667	31.19	0.0306
FATLEVEL*PH_TYPE	1	0.1410667	0.1410667	0.05	0.8506
FATLEVE*REP(PH_TYPE)	2	6.1771333	3.0885667		
Error	16	12.628333	0.789271		
Corrected Total	23	142.982533			

Table 101 -- Analysis of variance for raw patties myoglobin

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_TYPE	1	20.6570250	20.6570250	8.78	0.0975
REP(PH_TYPE)	2	4.7056250	2.3528125		
FATLEVEL	1	4.2025000	4.2025000	122.12	0.0081
FATLEVEL*PH_TYPE	1	0.1056250	0.1056250	3.07	0.2219
FATLEVE*REP(PH_TYPE)	2	0.0688250	0.0344125		
Error	8	0.3802000	0.0475250		
Corrected Total	15	30.1198000			

Table 102 -- Analysis of variance for raw patties metmyoglobin

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_TYPE	1	359.766056	359.766056	2.07	0.2870
REP(PH_TYPE)	2	347.965813	173.982906		
FATLEVEL	1	53.107656	53.107656	0.74	0.4798
FATLEVEL*PH_TYPE	1	0.178506	0.178506	0.00	0.9647
FATLEVE*REP(PH_TYPE)	2	143.139262	71.569631		
Error	8	14.540450	1.817556		
Corrected Total	15	918.697744			

Table 103 -- Analysis of variance for raw patties total reducing ability

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_TYPE	1	0.04667760	0.04667760	41.40	0.0001
Error	14	0.01578291	0.00112735		
Corrected Total	15	0.06246052			

Table 104 -- Analysis of variance for raw patties ORP

Source	DF	Anova SS	Mean Square	F Value	Pr > F
PH_TYPE	1	13894.4450	13894.4450	35.82	0.0010
Error	6	1.6933750	0.2822292		
Corrected Total	7	14.0686875			

Table 105 -- Analysis of variance for effect of 1% salt on myoglobin of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	6.4530281	6.4530281		
PH_GROUP	1	35.5535281	35.5535281	21.18	0.1362
PH_GROUP*BLOCK	1	1.6790281	1.6790281		
FATLEVEL	1	10.6375781	10.6375781	231.36	0.0043
FATLEVEL*PH_GROUP	1	0.2538281	0.2538281	5.52	0.1432
FATLEV*BLOCK(PH_GRO)	2	0.0919562	0.0459781		
TREATMEN	1	9.0206281	9.0206281	528.97	0.0001
PH_GROUP*TREATMEN	1	0.2161531	0.2161531	12.68	0.0236
FATLEVEL*TREATMEN	1	0.1313281	0.1313281	7.70	0.0501
FATLEV*PH_GRO*TREATM	1	0.0019531	0.0019531	0.11	0.7521
BLOC*TREA(FATL*PH_G)	4	0.0682125	0.0170531		
Error	16	0.7262500	0.0453906		
Corrected Total	31	64.8334719			

Table 106 -- Analysis of variance for effect of 1% salt on metmyoglobin of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	709.04365	709.04365		
PH_GROUP	1	2106.81633	2106.81633	92.82	0.0658
PH_GROUP*BLOCK	1	22.69695	22.69695		
FATLEVEL	1	97.26638	97.26638	0.42	0.5846
FATLEVEL*PH_GROUP	1	0.16388	0.16388	0.00	0.9813
FATLEV*BLOCK(PH_GRO)	2	466.46238	233.23119		
TREATMEN	1	884.83728	884.83728	128.84	0.0003
PH_GROUP*TREATMEN	1	363.89275	363.89275	52.99	0.0019
FATLEVEL*TREATMEN	1	0.19688	0.19688	0.03	0.8738
FATLEV*PH_GRO*TREATM	1	0.03713	0.03713	0.01	0.9449
BLOC*TREA(FATL*PH_G)	4	27.47074	6.86768		
Error	16	28.53825	1.78364		
Corrected Total	31	4707.42260			

Table 107 -- Analysis of variance for Hunter color "L" of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	29.70375	29.70375		
PH_GROUP	1	40.30042	40.30042	219.32	0.0429
PH_GROUP*BLOCK	1	0.18375	0.18375		
FATLEVEL	1	2105.62667	2105.62667	91.33	0.0108
FATLEVEL*PH_GROUP	1	1.12667	1.12667	0.05	0.8456
FATLEV*BLOCK(PH_GRO)	2	46.11000	23.05500		
TREATMEN	5	187.90083	37.58017	3.23	0.0268
PH_GROUP*TREATMEN	5	41.75083	8.35017	0.72	0.6175
FATLEVEL*TREATMEN	5	27.39208	5.47842	0.47	0.7934
FATLEV*PH_GRO*TREATM	5	51.57708	10.31542	0.89	0.5081
BLOC*TREA(FATL*PH_G)	20	232.61750	11.63088		
Error	48	392.21000	8.17104		
Corrected Total	95	3156.49958			

Table 108 -- Analysis of variance for Hunter color "a" of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	1.815000	1.815000		
PH_GROUP	1	68.006667	68.006667	4.00	0.2952
PH_GROUP*BLOCK	1	17.001667	17.001667		
FATLEVEL	1	0.041667	0.041667	0.04	0.8668
FATLEVEL*PH_GROUP	1	56.426667	56.426667	48.89	0.0198
FATLEV*BLOCK(PH_GRO)	2	2.308333	1.154167		
TREATMEN	5	815.730833	163.146167	38.68	0.0001
PH_GROUP*TREATMEN	5	154.213333	30.842667	7.31	0.0005
FATLEVEL*TREATMEN	5	23.933333	4.786667	1.13	0.3745
FATLEV*PH_GRO*TREATM	5	24.785833	4.957167	1.18	0.3558
BLOC*TREA(FATL*PH_G)	20	84.360000	4.218000		
Error	48	14.29000	0.29771		
Corrected Total	95	1262.91333			

Table 109 -- Analysis of variance for Hunter color "b" of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.960000	0.960000		
PH_GROUP	1	0.700417	0.700417	3.47	0.3135
PH_GROUP*BLOCK	1	0.201667	0.201667		
FATLEVEL	1	256.106667	256.106667	159.03	0.0062
FATLEVEL*PH_GROUP	1	31.740000	31.740000	19.71	0.0472
FATLEV*BLOCK(PH_GRO)	2	3.220833	1.610417		
TREATMEN	5	62.987083	12.597417	18.17	0.0001
PH_GROUP*TREATMEN	5	8.112083	1.622417	2.34	0.0793
FATLEVEL*TREATMEN	5	4.468333	0.893667	1.29	0.3076
FATLEV*PH_GRO*TREATM	5	5.150000	1.030000	1.49	0.2386
BLOC*TREA(FATL*PH_G)	20	13.862500	0.693125		
Error	48	3.120000	0.065000		
Corrected Total	95	390.629583			

Table 110 -- Analysis of variance for Hunter color "a/b" ratio of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.00050417	0.00050417		
PH_GROUP	1	0.96400417	0.96400417	4.12	0.2915
PH_GROUP*BLOCK	1	0.23403750	0.23403750		
FATLEVEL	1	4.69935000	4.69935000	3862.48	0.0003
FATLEVEL*PH_GROUP	1	0.01926667	0.01926667	15.84	0.0577
FATLEV*BLOCK(PH_GRO)	2	0.00243333	0.00121667		
TREATMEN	5	4.67516250	0.93503250	19.60	0.0001
PH_GROUP*TREATMEN	5	1.11787083	0.22357417	4.69	0.0054
FATLEVEL*TREATMEN	5	0.19632500	0.03926500	0.82	0.5480
FATLEV*PH_GRO*TREATM	5	0.26958333	0.05391667	1.13	0.3768
BLOC*TREA(FATL*PH_G)	20	0.95432500	0.04771625		
Error	48	0.5160000	0.0107500		
Corrected Total	95	13.6488625			

Table 111 -- Analysis of variance for Hunter color saturation index of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	1.599084	1.599084		
PH_GROUP	1	41.540859	41.540859	5.74	0.2517
PH_GROUP*BLOCK	1	7.232526	7.232526		
FATLEVEL	1	105.148134	105.148134	30.72	0.0310
FATLEVEL*PH_GROUP	1	75.917051	75.917051	22.18	0.0422
FATLEV*BLOCK(PH_GRO)	2	6.844777	3.422389		
TREATMEN	5	716.867568	143.373514	38.40	0.0001
PH_GROUP*TREATMEN	5	107.565109	21.513022	5.76	0.0019
FATLEVEL*TREATMEN	5	24.430634	4.886127	1.31	0.3001
FATLEV*PH_GRO*TREATM	5	27.555543	5.511109	1.48	0.2417
BLOC*TREA(FATL*PH_G)	20	74.679987	3.733999		
Error	48	6.20705	0.12931		
Corrected Total	95	1195.58832			

Table 112 -- Analysis of variance for Hunter color hue angle of raw patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	19.28730	19.28730		
PH_GROUP	1	635.56188	635.56188	4.07	0.2930
PH_GROUP*BLOCK	1	156.18753	156.18753		
FATLEVEL	1	2186.09138	2186.09138	888.55	0.0011
FATLEVEL*PH_GROUP	1	10.80713	10.80713	4.39	0.1711
FATLEV*BLOCK(PH_GRO)	2	4.92060	2.46030		
TREATMEN	5	2549.11525	509.82305	22.45	0.0001
PH_GROUP*TREATMEN	5	1005.05518	201.01104	8.85	0.0001
FATLEVEL*TREATMEN	5	37.67493	7.53499	0.33	0.8877
FATLEV*PH_GRO*TREATM	5	156.28196	31.25639	1.38	0.2750
BLOC*TREA(FATL*PH_G)	20	454.21990	22.71099		
Error	48	211.32305	4.40256		
Corrected Total	95	7426.52607			

Table 113 -- Analysis of variance for initial weight of patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	252.01562	252.01562		
PH_GROUP	1	2401.81674	2401.81674	776.85	0.0228
PH_GROUP*BLOCK	1	3.09174	3.09174		
FATLEVEL	1	562.08507	562.08507	152.80	0.0065
FATLEVEL*PH_GROUP	1	0.39063	0.39063	0.11	0.7755
FATLEV*BLOCK(PH_GRO)	2	7.35736	3.67868		
TREATMEN	5	1663.79312	332.75862	24.41	0.0001
PH_GROUP*TREATMEN	5	399.10201	79.82040	5.85	0.0017
FATLEVEL*TREATMEN	5	36.01035	7.20207	0.53	0.7523
FATLEV*PH_GRO*TREATM	5	66.14146	13.22829	0.97	0.4595
BLOC*TREA(FATL*PH_G)	20	272.68694	13.63435		
Error	96	1158.39333	12.06660		
Corrected Total	143	6822.88437			

Table 114 -- Analysis of variance for cooking time of patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	1640.2500	1640.2500		
PH_GROUP	1	3520.4444	3520.4444	1.95	0.3957
PH_GROUP*BLOCK	1	1806.2500	1806.2500		
FATLEVEL	1	1034.6944	1034.6944	2.16	0.2796
FATLEVEL*PH_GROUP	1	2256.2500	2256.2500	4.70	0.1623
FATLEV*BLOCK(PH_GRO)	2	959.2222	479.6111		
TREATMEN	5	3297.6667	659.5333	0.61	0.6958
PH_GROUP*TREATMEN	5	10442.7222	2088.5444	1.92	0.1357
FATLEVEL*TREATMEN	5	5230.8056	1046.1611	0.96	0.4640
FATLEV*PH_GRO*TREATM	5	8105.0833	1621.0167	1.49	0.2371
BLOC*TREA(FATL*PH_G)	20	21746.2778	1087.3139		
Error	96	104129.3333	1084.6806		
Corrected Total	143	164169.0000			

Table 115 -- Analysis of variance for cooked yield of patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	109.01100	109.01100		
PH_GROUP	1	2421.87016	2421.87016	75.53	0.0729
PH_GROUP*BLOCK	1	32.06391	32.06391		
FATLEVEL	1	82.52208	82.52208	1.30	0.3719
FATLEVEL*PH_GROUP	1	119.92075	119.92075	1.89	0.3026
FATLEV*BLOCK(PH_GRO)	2	126.66990	63.33495		
TREATMEN	5	1472.80275	294.56055	5.24	0.0031
PH_GROUP*TREATMEN	5	886.72780	177.34556	3.16	0.0293
FATLEVEL*TREATMEN	5	277.93929	55.58786	0.99	0.4490
FATLEV*PH_GRO*TREATM	5	397.75614	79.55123	1.42	0.2614
BLOC*TREA(FATL*PH_G)	20	1124.04304	56.20215		
Error	96	4902.55727	51.06830		
Corrected Total	143	11953.88408			

Table 116 -- Analysis of variance for diameter shrinkage of patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	3.1901042	3.1901042		
PH_GROUP	1	76.1484375	76.1484375	4.46	0.2816
PH_GROUP*BLOCK	1	17.0859375	17.0859375		
FATLEVEL	1	52.6584375	52.6584375	25.45	0.0371
FATLEVEL*PH_GROUP	1	4.5501042	4.5501042	2.20	0.2763
FATLEV*BLOCK(PH_GRO)	2	4.1385417	2.0692708		
TREATMEN	5	69.8771875	13.9754375	10.68	0.0001
PH_GROUP*TREATMEN	5	43.0246875	8.6049375	6.57	0.0009
FATLEVEL*TREATMEN	5	5.5371875	1.1074375	0.85	0.5333
FATLEV*PH_GRO*TREATM	5	6.9655208	1.3931042	1.06	0.4091
BLOC*TREA(FATL*PH_G)	20	26.1779167	1.3088958		
Error	48	31.045000	0.646771		
Corrected Total	95	340.399063			

Table 117 -- Analysis of variance for thickness expansion of patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	504.16667	504.16667		
PH_GROUP	1	1837.50000	1837.50000	49.00	0.0903
PH_GROUP*BLOCK	1	37.50000	37.50000		
FATLEVEL	1	816.66667	816.66667	19.60	0.0474
FATLEVEL*PH_GROUP	1	266.66667	266.66667	6.40	0.1271
FATLEV*BLOCK(PH_GRO)	2	83.33333	41.66667		
TREATMEN	5	250.00000	50.00000	0.60	0.7026
PH_GROUP*TREATMEN	5	575.00000	115.00000	1.37	0.2761
FATLEVEL*TREATMEN	5	70.83333	14.16667	0.17	0.9710
FATLEV*PH_GRO*TREATM	5	245.83333	49.16667	0.59	0.7098
BLOC*TREA(FATL*PH_G)	20	1675.00000	83.75000		
Error	48	1200.00000	25.00000		
Corrected Total	95	7562.50000			

Table 118 -- Analysis of variance for penetrometer load of patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	356240.7	356240.7		
PH_GROUP	1	7078548.2	7078548.2	9051.37	0.0067
PH_GROUP*BLOCK	1	782.0	782.0		
FATLEVEL	1	262504.2	262504.2	13.29	0.0677
FATLEVEL*PH_GROUP	1	77407.0	77407.0	3.92	0.1863
FATLEV*BLOCK(PH_GRO)	2	39506.7	19753.4		
TREATMEN	5	10615898.6	2123179.7	137.53	0.0001
PH_GROUP*TREATMEN	5	606329.7	121265.9	7.85	0.0003
FATLEVEL*TREATMEN	5	389410.7	77882.1	5.04	0.0038
FATLEV*PH_GRO*TREATM	5	330798.6	66159.7	4.29	0.0082
BLOC*TREA(FATL*PH_G)	20	308765.6	15438.3		
Error	48	343867.0	7163.9		
Corrected Total	95	20410059.0			

Table 119 -- Analysis of variance for external Hunter color "L" of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	1.03361	1.03361		
PH_GROUP	1	450.85444	450.85444	14.64	0.1628
PH_GROUP*BLOCK	1	30.80250	30.80250		
FATLEVEL	1	178.22250	178.22250	14.27	0.0635
FATLEVEL*PH_GROUP	1	10.56250	10.56250	0.85	0.4548
FATLEV*BLOCK(PH_GRO)	2	24.97111	12.48556		
TREATMEN	5	1139.46583	227.89317	21.88	0.0001
PH_GROUP*TREATMEN	5	23.34806	4.66961	0.45	0.8095
FATLEVEL*TREATMEN	5	54.28167	10.85633	1.04	0.4206
FATLEV*PH_GRO*TREATM	5	57.65167	11.53033	1.11	0.3879
BLOC*TREA(FATL*PH_G)	20	208.35278	10.41764		
Error	96	406.13333	4.23056		
Corrected Total	143	2585.68000			

Table 120 -- Analysis of variance for external Hunter color "a" of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	4.2367361	4.2367361		
PH_GROUP	1	2.9184028	2.9184028	0.56	0.5922
PH_GROUP*BLOCK	1	5.2517361	5.2517361		
FATLEVEL	1	1.6256250	1.6256250	0.20	0.6984
FATLEVEL*PH_GROUP	1	19.1406250	19.1406250	2.36	0.2646
FATLEV*BLOCK(PH_GRO)	2	16.2490278	8.1245139		
TREATMEN	5	52.7806250	10.5561250	3.55	0.0186
PH_GROUP*TREATMEN	5	2.4695139	0.4939028	0.17	0.9722
FATLEVEL*TREATMEN	5	13.6139583	2.7227917	0.91	0.4915
FATLEV*PH_GRO*TREATM	5	32.1722917	6.4344583	2.16	0.0996
BLOC*TREA(FATL*PH_G)	20	59.5375000	2.9768750		
Error	96	134.873333	1.404931		
Corrected Total	143	344.869375			

Table 121 -- Analysis of variance for external Hunter color "b" of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.080278	0.080278		
PH_GROUP	1	122.471111	122.471111	101.22	0.0631
PH_GROUP*BLOCK	1	1.210000	1.210000		
FATLEVEL	1	13.201111	13.201111	5.23	0.1495
FATLEVEL*PH_GROUP	1	1.913611	1.913611	0.76	0.4758
FATLEV*BLOCK(PH_GRO)	2	5.050278	2.525139		
TREATMEN	5	57.625556	11.525111	12.12	0.0001
PH_GROUP*TREATMEN	5	11.539722	2.307944	2.43	0.0712
FATLEVEL*TREATMEN	5	4.758056	0.951611	1.00	0.4429
FATLEV*PH_GRO*TREATM	5	5.823889	1.164778	1.22	0.3342
BLOC*TREA(FATL*PH_G)	20	19.026111	0.951306		
Error	96	134.346667	1.399444		
Corrected Total	143	377.046389			

Table 122 -- Analysis of variance for external Hunter color "a/b" ratio of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.03240000	0.03240000		
PH_GROUP	1	0.03180278	0.03180278	0.65	0.5687
PH_GROUP*BLOCK	1	0.04913611	0.04913611		
FATLEVEL	1	0.00004444	0.00004444	0.00	0.9871
FATLEVEL*PH_GROUP	1	0.25840278	0.25840278	1.92	0.2997
FATLEV*BLOCK(PH_GRO)	2	0.26856944	0.13428472		
TREATMEN	5	0.86138889	0.17227778	4.36	0.0076
PH_GROUP*TREATMEN	5	0.09141389	0.01828278	0.46	0.7995
FATLEVEL*TREATMEN	5	0.17712222	0.03542444	0.90	0.5027
FATLEV*PH_GRO*TREATM	5	0.41624722	0.08324944	2.11	0.1070
BLOC*TREA(FATL*PH_G)	20	0.79079444	0.03953972		
Error	96	2.27806667	0.02372986		
Corrected Total	143	5.25538889			

Table 123 -- Analysis of variance for external Hunter color saturation index of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.612306	0.612306		
PH_GROUP	1	124.824756	124.824756	437.47	0.0304
PH_GROUP*BLOCK	1	0.285334	0.285334		
FATLEVEL	1	15.635434	15.635434	10.93	0.0806
FATLEVEL*PH_GROUP	1	0.009834	0.009834	0.01	0.9415
FATLEV*BLOCK(PH_GRO)	2	2.861313	1.430656		
TREATMEN	5	44.932612	8.986522	13.23	0.0001
PH_GROUP*TREATMEN	5	8.811856	1.762371	2.59	0.0578
FATLEVEL*TREATMEN	5	4.576095	0.915219	1.35	0.2856
FATLEV*PH_GRO*TREATM	5	1.861845	0.372369	0.55	0.7379
BLOC*TREA(FATL*PH_G)	20	13.587831	0.679392		
Error	96	85.103133	0.886491		
Corrected Total	143	303.102349			

Table 124 -- Analysis of variance for external Hunter color hue angle of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	83.60054	83.60054		
PH_GROUP	1	74.56323	74.56323	0.51	0.6040
PH_GROUP*BLOCK	1	145.04188	145.04188		
FATLEVEL	1	0.79804	0.79804	0.00	0.9655
FATLEVEL*PH_GROUP	1	675.65338	675.65338	2.02	0.2910
FATLEV*BLOCK(PH_GRO)	2	668.42261	334.21130		
TREATMEN	5	2232.91356	446.58271	4.36	0.0076
PH_GROUP*TREATMEN	5	227.73189	45.54638	0.44	0.8121
FATLEVEL*TREATMEN	5	479.55382	95.91076	0.94	0.4788
FATLEV*PH_GRO*TREATM	5	1098.93441	219.78688	2.15	0.1016
BLOC*TREA(FATL*PH_G)	20	2048.75744	102.43787		
Error	96	5544.78213	57.75815		
Corrected Total	143	13280.75293			

Table 125 -- Analysis of variance for internal Hunter color "L" of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	48.30250	48.30250		
PH_GROUP	1	1896.60250	1896.60250	153.36	0.0513
PH_GROUP*BLOCK	1	12.36694	12.36694		
FATLEVEL	1	262.98028	262.98028	55.66	0.0175
FATLEVEL*PH_GROUP	1	2.94694	2.94694	0.62	0.5124
FATLEV*BLOCK(PH_GRO)	2	9.44944	4.72472		
TREATMEN	5	855.69889	171.13978	20.82	0.0001
PH_GROUP*TREATMEN	5	110.46500	22.09300	2.69	0.0515
FATLEVEL*TREATMEN	5	55.62389	11.12478	1.35	0.2833
FATLEV*PH_GRO*TREATM	5	35.81556	7.16311	0.87	0.5176
BLOC*TREA(FATL*PH_G)	20	164.41444	8.22072		
Error	96	124.12000	1.29292		
Corrected Total	143	3578.78639			

Table 126 -- Analysis of variance for internal Hunter color "a" of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	9.610000	9.610000		
PH_GROUP	1	86.800278	86.800278	5.61	0.2543
PH_GROUP*BLOCK	1	15.471111	15.471111		
FATLEVEL	1	6.502500	6.502500	1.78	0.3136
FATLEVEL*PH_GROUP	1	3.062500	3.062500	0.84	0.4563
FATLEV*BLOCK(PH_GRO)	2	7.297778	3.648889		
TREATMEN	5	216.300833	43.260167	23.18	0.0001
PH_GROUP*TREATMEN	5	16.121389	3.224278	1.73	0.1743
FATLEVEL*TREATMEN	5	5.355833	1.071167	0.57	0.7193
FATLEV*PH_GRO*TREATM	5	7.864167	1.572833	0.84	0.5354
BLOC*TREA(FATL*PH_G)	20	37.331111	1.866556		
Error	96	52.420000	0.546042		
Corrected Total	143	464.137500			

Table 127 -- Analysis of variance for internal Hunter color "b" of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	4.168403	4.168403		
PH_GROUP	1	187.005625	187.005625	7459.50	0.0074
PH_GROUP*BLOCK	1	0.025069	0.025069		
FATLEVEL	1	6.208403	6.208403	1.25	0.3801
FATLEVEL*PH_GROUP	1	0.918403	0.918403	0.18	0.7093
FATLEV*BLOCK(PH_GRO)	2	9.949028	4.974514		
TREATMEN	5	24.507292	4.901458	4.10	0.0101
PH_GROUP*TREATMEN	5	38.798958	7.759792	6.49	0.0010
FATLEVEL*TREATMEN	5	6.696181	1.339236	1.12	0.3818
FATLEV*PH_GRO*TREATM	5	6.767847	1.353569	1.13	0.3761
BLOC*TREA(FATL*PH_G)	20	23.925833	1.196292		
Error	96	40.533333	0.422222		
Corrected Total	143	349.504375			

Table 128 -- Analysis of variance for internal Hunter color "a/b" ratio of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.18561736	0.18561736		
PH_GROUP	1	3.28515625	3.28515625	38.84	0.1013
PH_GROUP*BLOCK	1	0.08458403	0.08458403		
FATLEVEL	1	0.29793403	0.29793403	1.90	0.3022
FATLEVEL*PH_GROUP	1	0.16335069	0.16335069	1.04	0.4149
FATLEV*BLOCK(PH_GRO)	2	0.31386806	0.15693403		
TREATMEN	5	3.25051458	0.65010292	19.11	0.0001
PH_GROUP*TREATMEN	5	0.81886458	0.16377292	4.82	0.0047
FATLEVEL*TREATMEN	5	0.26555347	0.05311069	1.56	0.2163
FATLEV*PH_GRO*TREATM	5	0.32133681	0.06426736	1.89	0.1413
BLOC*TREA(FATL*PH_G)	20	0.68024722	0.03401236		
Error	96	1.32366667	0.01378819		
Corrected Total	143	10.99069375			

Table 129 -- Analysis of variance for internal Hunter color saturation index of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.7847007	0.7847007		
PH_GROUP	1	72.7466840	72.7466840	72.38	0.0745
PH_GROUP*BLOCK	1	1.0050062	1.0050062		
FATLEVEL	1	0.3813063	0.3813063	0.29	0.6420
FATLEVEL*PH_GROUP	1	0.2558674	0.2558674	0.20	0.7003
FATLEV*BLOCK(PH_GRO)	2	2.5935847	1.2967924		
TREATMEN	5	57.8691618	11.5738324	10.66	0.0001
PH_GROUP*TREATMEN	5	17.0735618	3.4147124	3.14	0.0296
FATLEVEL*TREATMEN	5	3.2046062	0.6409212	0.59	0.7076
FATLEV*PH_GRO*TREATM	5	4.1015285	0.8203057	0.76	0.5922
BLOC*TREA(FATL*PH_G)	20	21.7204917	1.0860246		
Error	96	28.141933	0.293145		
Corrected Total	143	209.878433			

Table 130 -- Analysis of variance for internal Hunter color hue angle of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	405.48534	405.48534		
PH_GROUP	1	6303.30138	6303.30138	22.03	0.1336
PH_GROUP*BLOCK	1	286.17361	286.17361		
FATLEVEL	1	368.76801	368.76801	1.44	0.3525
FATLEVEL*PH_GROUP	1	158.84401	158.84401	0.62	0.5130
FATLEV*BLOCK(PH_GRO)	2	510.93496	255.46748		
TREATMEN	5	6508.57060	1301.71412	20.46	0.0001
PH_GROUP*TREATMEN	5	1030.02345	206.00469	3.24	0.0266
FATLEVEL*TREATMEN	5	262.63420	52.52684	0.83	0.5463
FATLEV*PH_GRO*TREATM	5	386.79718	77.35944	1.22	0.3380
BLOC*TREA(FATL*PH_G)	20	1272.61716	63.63086		
Error	96	2261.2669	23.5549		
Corrected Total	143	19755.4168			

Table 131 -- Analysis of variance for pH of cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	0.0190005	0.0190005		
PH_GROUP	1	16.7737630	16.7737630	4065.85	0.0100
PH_GROUP*BLOCK	1	0.0041255	0.0041255		
FATLEVEL	1	0.1758130	0.1758130	17.51	0.0526
FATLEVEL*PH_GROUP	1	0.1235255	0.1235255	12.31	0.0725
FATLEV*BLOCK(PH_GRO)	2	0.0200760	0.0100380		
TREATMEN	5	42.5337276	8.5067455	1241.54	0.0001
PH_GROUP*TREATMEN	5	0.4511901	0.0902380	13.17	0.0001
FATLEVEL*TREATMEN	5	0.5548401	0.1109680	16.20	0.0001
FATLEV*PH_GRO*TREATM	5	0.0643026	0.0128605	16.20	0.0001
BLOC*TREA(FATL*PH_G)	20	0.1370354	0.0068518		
Error	144	0.1104250	0.0007668		
Corrected Total	191	60.9678245			

Table 132 -- Analysis of variance for undenatured myoglobin in cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	6.5783010	6.5783010		
PH_GROUP	1	70.0245844	70.0245844	1538.97	0.0162
PH_GROUP*BLOCK	1	0.0455010	0.0455010		
FATLEVEL	1	0.5750510	0.5750510	0.29	0.6456
FATLEVEL*PH_GROUP	1	0.3185510	0.3185510	0.16	0.7285
FATLEV*BLOCK(PH_GRO)	2	4.0045521	2.0022760		
TREATMEN	5	69.9456677	13.9891335	61.25	0.0001
PH_GROUP*TREATMEN	5	8.8393344	1.7678669	7.74	0.0003
FATLEVEL*TREATMEN	5	1.4543927	0.2908785	1.27	0.3139
FATLEV*PH_GRO*TREATM	5	1.3598427	0.2719685	1.19	0.3489
BLOC*TREA(FATL*PH_G)	20	4.5680708	0.2284035		
Error	48	1.022850	0.021309		
Corrected Total	95	168.736699			

Table 133 -- Analysis of variance for metmyoglobin in cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	704.70844	704.70844		
PH_GROUP	1	252.98027	252.98027	1.92	0.3980
PH_GROUP*BLOCK	1	131.78907	131.78907		
FATLEVEL	1	872.90282	872.90282	9.49	0.0912
FATLEVEL*PH_GROUP	1	64.78020	64.78020	0.70	0.4896
FATLEV*BLOCK(PH_GRO)	2	183.90120	91.95060		
TREATMEN	5	3166.01382	633.20276	27.27	0.0001
PH_GROUP*TREATMEN	5	2311.59878	462.31976	19.91	0.0001
FATLEVEL*TREATMEN	5	211.34966	42.26993	1.82	0.1545
FATLEV*PH_GRO*TREATM	5	400.03295	80.00659	3.45	0.0208
BLOC*TREA(FATL*PH_G)	20	464.40939	23.22047		
Error	48	107.52340	2.24007		
Corrected Total	95	8871.99000			

Table 134 -- Analysis of variance for percent myoglobin denaturation in cooked patties

Source	DF	Anova SS	Mean Square	F Value	Pr > F
BLOCK	1	3482.6913	3482.6913		
PH_GROUP	1	5120.2209	5120.2209	533.98	0.0275
PH_GROUP*BLOCK	1	9.5887	9.5887		
FATLEVEL	1	406.7267	406.7267	1.51	0.3446
FATLEVEL*PH_GROUP	1	0.6468	0.6468	0.00	0.9654
FATLEV*BLOCK(PH_GRO)	2	540.2250	270.1125		
TREATMEN	5	11318.5516	2263.7103	42.49	0.0001
PH_GROUP*TREATMEN	5	556.0566	111.2113	2.09	0.1095
FATLEVEL*TREATMEN	5	324.3527	64.8705	1.22	0.3371
FATLEV*PH_GRO*TREATM	5	194.4413	38.8883	0.73	0.6093
BLOC*TREA(FATL*PH_G)	20	1065.5158	53.2758		
Error	48	228.4426	4.7592		
Corrected Total	95	23247.4601			

Table 135 -- Analysis of variance for sensory texture of cooked patties

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BLOCK	1	0.058011	0.058011		
PH_GROUP	1	51.100443	51.100443	77233.46	0.0023
PH_GROUP*BLOCK	1	0.000662	0.000662		
FATLEVEL	1	204.602411	204.602411	824.09	0.0012
FATLEVEL*PH_GROUP	1	0.613654	0.613654	2.47	0.2565
FATLEV*BLOCK(PH_GRO)	2	0.496555	0.248278		
TREATMEN	5	206.661034	41.332207	19.40	0.0001
PH_GROUP*TREATMEN	5	63.812243	12.762449	5.99	0.0015
FATLEVEL*TREATMEN	5	24.318175	4.863635	2.28	0.0853
FATLEV*PH_GRO*TREATM	5	2.733847	0.546769	0.26	0.9314
BLOC*TREA(FATL*PH_G)	20	42.605433	2.130272		
Error	678	840.895833	1.240259		
Corrected Total	725	1439.333333			

Table 136 -- Analysis of variance for sensory juiciness of cooked patties

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BLOCK	1	23.271921	23.271921		
PH_GROUP	1	0.888063	0.888063	1.41	0.4452
PH_GROUP*BLOCK	1	0.628396	0.628396		
FATLEVEL	1	77.923365	77.923365	6.06	0.1328
FATLEVEL*PH_GROUP	1	0.035876	0.035876	0.00	0.9627
FATLEV*BLOCK(PH_GRO)	2	25.699530	12.849765		
TREATMEN	5	125.863950	25.172790	9.47	0.0001
PH_GROUP*TREATMEN	5	56.036316	11.207263	4.21	0.0089
FATLEVEL*TREATMEN	5	2.338840	0.467768	0.18	0.9685
FATLEV*PH_GRO*TREATM	5	17.301449	3.460290	1.30	0.3029
BLOC*TREA(FATL*PH_G)	20	53.184452	2.659223		
Error	678	1114.429167	1.643701		
Corrected Total	725	1498.000000			

Table 137 -- Analysis of variance for beef flavor of cooked patties

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BLOCK	1	0.358355	0.358355		
PH_GROUP	1	96.824497	96.824497	39.21	0.1008
PH_GROUP*BLOCK	1	2.469291	2.469291		
FATLEVEL	1	3.378150	3.378150	29.34	0.0324
FATLEVEL*PH_GROUP	1	0.352778	0.352778	3.06	0.2222
FATLEV*BLOCK(PH_GRO)	2	0.230315	0.115157		
TREATMEN	5	261.451530	52.290306	34.08	0.0001
PH_GROUP*TREATMEN	5	27.560864	5.512173	3.59	0.0176
FATLEVEL*TREATMEN	5	5.449432	1.089886	0.71	0.6227
FATLEV*PH_GRO*TREATM	5	3.503222	0.700644	0.46	0.8036
BLOC*TREA(FATL*PH_G)	20	30.685578	1.534279		
Error	678	1179.483333	1.739651		
Corrected Total	725	1611.858127			

Table 138 -- Analysis of variance for off-flavor of cooked patties

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BLOCK	1	1.63012	1.63012		
PH_GROUP	1	7.97935	7.97935	3648.16	0.0105
PH_GROUP*BLOCK	1	0.00219	0.00219		
FATLEVEL	1	0.00492	0.00492	0.00	0.9713
FATLEVEL*PH_GROUP	1	0.13307	0.13307	0.04	0.8526
FATLEV*BLOCK(PH_GRO)	2	5.98917	2.99459		
TREATMEN	5	1089.09816	217.81963	113.78	0.0001
PH_GROUP*TREATMEN	5	17.05275	3.41055	1.78	0.1625
FATLEVEL*TREATMEN	5	2.94903	0.58981	0.31	0.9023
FATLEV*PH_GRO*TREATM	5	12.65349	2.53070	1.32	0.2950
BLOC*TREA(FATL*PH_G)	20	38.28657	1.91433		
Error	678	1656.99167	2.44394		
Corrected Total	725	2831.18457			

APPENDIX G.

EXAMPLE OF SAS PROGRAM FOR SPLIT-SPLIT PLOT DESIGN

```
options ls = 75 ps = 60;
Title1 'PMD IN BEEF PATTIES WITH BROWNING AGENTS';

libname lib 'disk$usr7:[h.slh5c.sas.dis]';
data lib.lfcpigtr;
infile '[h.slh5c.sas.dis]lfcpigtr.dat';
input FatLevel $ 1-7 pH_Group $ 8-13 Block 14 Treatmen $ 15-22 Mb 23-26 .2
MetMb 27-31 .2 PMD 32-36 .2 Raw_Mb 37-41 .2;

proc print;
run;

proc ANOVA;
class FatLevel pH_Group Block Treatmen;

model Mb MetMb PMD Raw_Mb = Block pH_Group pH_Group*Block

FatLevel
FatLevel*pH_Group
FatLevel*Block(pH_Group)

Treatmen
pH_Group*Treatmen
FatLevel*Treatmen
pH_Group*FatLevel*Treatmen
Treatmen*Block(pH_Group FatLevel);

Test H = pH_Group E = pH_Group*Block;
Test H = FatLevel FatLevel*pH_Group E = FatLevel*Block(pH_Group);
Test H = Treatmen pH_Group*Treatmen FatLevel*Treatmen
pH_Group*FatLevel*Treatmen E = Treatmen*Block(pH_Group FatLevel);

means pH_Group / LSD E = pH_Group*Block;
means FatLevel / LSD E = FatLevel*Block(pH_Group);
means Treatmen / LSD E = Treatmen*Block(pH_Group FatLevel);

means pH_Group FatLevel Treatmen FatLevel*pH_Group pH_Group*Treatmen
FatLevel*Treatmen pH_Group*FatLevel*Treatmen;
run;
```

CURRICULUM VITAE

IGOR V. MOISEEV

OBJECTIVES: To obtain a middle management position in R&D, QC/QA at food company or research scientist position in food science & technology with growth and mobility potential.

SUMMARY: One year of teaching graduate-level courses. Three years of research in academic institute involving various areas of food chemistry, microbiology, and technology. Two years of army service. One year of work in food industry.

PROFESSIONAL EXPERIENCE:

GRADUATE RESEARCH AND TEACHING ASSISTANT,
Department of Nutrition and Food Sciences (NFS),
Utah State University (USU), Logan, Utah, 1991 -1997.

Responsibilities: Accomplishing research in area of new product development and quality control of meat products under direction of Dr. Cornforth, D. P. Application of non-meat ingredients (phosphates, soy protein isolates, whey protein concentrates) for improvement quality, texture, taste, and shelf-life of meat products (roast beef, hamburger, turkey roll). Chemical, physical, microbial, and sensory testing of meat products. *Teaching:* Food Analysis(550/650) and Food Chemistry (557/657) laboratory sections. *Quality management:* basic knowledge of TQM and ISO 9000.

RESEARCH ASSOCIATE - II, I,

Department of Quality Control, Laboratory of Chemical & Physical Methods, All-Union Research Institute of Fruit and Vegetable Canned-Drying Industry (AURIFVCDI), Vidnoe, Moscow region, Russia 1987-1991.

Responsibilities: Development of analytical test methods and standards of identity for quality control and safety of canned fruit-vegetable products by using GLC/MS, HPLC, UV-VIS and AA spectrometry. Developed express GC method of determination ethyl alcohol in fruit juices and liquor, based on catalyzed combustion of alcohol vapor. 25% extension/consulting travel to QC/R&D labs at fruit/vegetable canning plants in ex-Soviet Union republics.

FREELANCE TECHNICAL TRANSLATOR (English-Russian),

Department of Food Chemistry, All-Union Institute of Scientific & Technical Information, Moscow, Russia, 1989-1991.

Responsibilities: Summarized and translated food chemistry scientific articles (about 500 articles) from English into Russian for publishing in National Abstract Journal.

QUALITY CONTROL OFFICER,
Department of Meat & Dairy Products, Foreign Trade Division of the USSR, Moscow, Russia, 1984-1985.

Responsibilities: Certification, quantity and quality expertise of the imported meat and dairy products.

MILITARY SERVICE:

LIEUTENANT, SERGEANT, PRIVATE,
Military School of Engineering, Tapa, Estonia, 1985-1987.

Responsibilities: Served as a cadet, mechanic-driver, instructor of practical driving of heavy artillery tractor. Taught cadets (30) how to maintain and drive artillery tractor. Managed (10 people) an army concrete plant. Discharged in rank of lieutenant in November, 1987.

EDUCATION:

Ph.D., Food Science & Human Nutrition (meat), USU, Logan, UT, 1997.
M.S., Food Science & Human Nutrition (meat/dairy), USU, Logan, UT, 1994.
B.S., Food Commodities Expertise & Organization of Retail Sales (fruits/vegetables/wine), Plekhanov All-Russian Economic Academy (PAREA), Moscow (MSK), Russia (RU), 1985.

SPECIAL SKILLS:

Computer knowledge: IBM, Mac, VMS; DOS & Windows, System 7, Internet, desktop publishing, spreadsheets; Statistica, StatView, SAS, Minitab, Basic programming. *Foreign languages:* Russian (professional level).

CONTINUING EDUCATION:

* Agricultural Extension Short Course, USU, Logan, UT, 1995.
* HACCP workshop, Washington State University, Pullman, WA, 1994.
* Processed meat workshop, University of Nebraska, Lincoln, NE, 1993.
* Teaching assistant workshop, USU, Logan, UT, 1991.
* Intensive English Language Program, USU, Logan, UT, 1991.
* Food Sci. courses, All-Russian Institute of Food Industry, Moscow, RU, 1989.
* Course of lecturer, Institute of Marxism, Vidnoe, RU, 1989-90.
* Professional photography course, Moscow 1988.
* Intensive Lieutenant program, School of Soviet Army Reserve, Kaliningrad, RU, 1987.

OTHER EXPERIENCES:

* Interpreter/guide for visiting Belarus agricultural scientists, USU, Logan, UT, 1995.
* Freelance Russian translator for T. J. Payne Market Develop., CA, 1993.
* Interpreter and guide for visiting Armenian group of dairymen, USU, 1992.
* Dept. group leader of agriculture labor union, AURIFVCDI, Vidnoe, RU, 1987-88.
* Member of summer athletic team (8-th place), PAREA, Moscow, RU, 1981-85.

HONORS:

* The Honor Society of PHI KAPPA PHI, USU, Logan, UT, 1995.
* Research Assistantship, NFS Dept., USU, Logan, UT, 1991-96.
* National Dean's List; USU Honor Roll, three quarters, 1992-93.
* Honor BS diploma (GPA = 3.95; rank 3-rd/100), PAREA, Moscow, Russia.

PROFESSIONAL AFFILIATIONS:

* Institute of Food Technologists.
* American Meat Science Association.
* American Translators Association.
* Publications - 4; Abstracts and Presentations - 6; Technical Reports - 4; Funded Research - 4.